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THE BARREL OF THE PELAGIC AMPHIPOD *PHRONZMA SEDENTARIA* (Forsk.) (CRUSTACEA: HYPERIIDEA)

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Abstract: Several pelagic animals have been postulated as the source of the transparent 'barrel' in which *Phronima sedentaria* (Forsk.) lives. It has been commonly held, although not on good evidence, that they are derived from tunicates. Many barrels, however, do not bear a close resemblance with the group most frequently proposed, pyrosomes. To clarify this zoological association, a sample of 70 barrels was submitted to data analysis. Principal coordinate analysis combining quantitative and qualitative characters disclosed six groups of barrels. These groups were subsequently thoroughly examined, and evidence found that they could only come from salps and pyrosomes. Observations on living animals are reported which support this conclusion. It is shown how salps and pyrosomes could be transformed into barrels, and a short discussion of what is thought to be a borderline case of host-parasite relationships is given.

INTRODUCTION

Phronimids are known to live in 'barrels'. These houses are made from the transparent test of pelagic animals, open at both ends and shaped by the amphipod. In some species, such as *Phronima curvipes* (Laval, 1968) and P. *colletti* (Chun, 1889, 1895; Vosseler, 1901; Laval, 1968), the barrel is shaped from a siphonophore which retains most of its appearance. But in other species, like *P. sedentaria* and *P. atlantica*, the 'host' is often so transformed that one can only make suggestions about its zoological origin.

Long ago Delle Chiaje (1841) described three kinds of barrels of *P. sedentaria,* differing in their ornamentation. He gave them specific names: *Doliolum mediterraneum* (for the form with smooth outer wall), D. *papillosum* (with tubercles), and *D. sulcatum* (with longitudinal ridges). Dudich (1926) later pointed out that the generic name *Doliolum* had been applied previously to the doliolid genus *Doliolum* Quoy & Gaimard 1834 (which has taxonomic precedence). Despite this, confusion persists and doliolids are still erroneously designated in some recent papers as possible hosts for phronimids.

Several zoologists, mainly at the end of the last century, tried in various ways to discover the origin of phronimid houses. Pagenstecher (1861) made histological sections of the wall of barrels of *Phronima sedentaria.* He found elongated cells embedded in an intercellular material reminiscent of the structure of the salp tunic. Nevertheless, he dismissed this possible origin because the tunic of salps is thinner

and less rigid. As further proof he indicated that salps do not possess the spiral filaments he found in the wall of barrels. Pagenstecher also eliminated appendicularian houses, medusae, the ctenophore Beroe, and heteropods as sources, because they are different from barrels in histological sections. Curiously he did not consider the possibility of pyrosomes as candidates.

Claus (1862, 1872) found three barrels which appeared to be transformed pyrosomes and, which he considered a definitive proof, a female Phronima sedentaria within a pyrosome not open at the apex, with some zooids remaining. He postulated that the ornamentation of tubercles and ridges appeared because of the amphipod action; they could be smoothed out later on. A comparison of many barrels led him to claim that a pyrosomian origin could account for all barrels.

Mayer (1879) did not dispute the suggestion that most houses came from pyrosomes, but mentioned a Phronima in a barrel with the appendage and nucleus typical of a salp. He also found a Phronima in the siphonophore Abyla pentagona, and observed in the laboratory the transformation of this siphonophore into a barrel. The same Phronima was then seen making a barrel from *Salpa fusiformis*. He did not give any specific name for this Phronima, but it could not have been P. sedentaria (Laval, 1968). No barrel could be obtained in the laboratory from the heteropod Pterotrachea, and further, chemical assays showed that the largest houses (perhaps associated with Phronima sedentaria) could only come from tunicates.

In 1889 and 1895, Chun reported the capture of P. diogenes (= P. colletfi) in siphonophores. Minkiewicz (1909) made extensive observations on the behaviour of P. sedentaria in its house but did not give any new information on barrel origin.

Dudich (1926) published an important study, mostly on P. atlantica (he found only three P. sedentaria with barrels). He described the outer surface of the barrel as being usually smooth, with occasional polygonal ornamentations, and observed 12 specimens with 6–7 longitudinal ridges. The analysis for tunicine in more than 50 barrels was always positive. Dudich concluded that the houses came only from pyrosomes – apparently unaware that salps also have tunicine. He stated, however, that there was no clear correlation between Phronima and pyrosome distributions.

Legendre (1940) found many barrels in the stomach contents of tuna. He remarked that they were so transformed, so scraped, and rubbed so smooth that the animal from which they had come was not recognizable. The outer wall was either corrugated in a way which could represent the location of pyrosome zooids, or with longitudinal stripes transversally striated, reminiscent of ctenophores.

In a book by Fraser (1962) a barrel photographed with a P. sedentaria is said, without any comment, to come from the salp *Iasis zonaria*. Some transverse stripes are apparent in the photograph (not well reproduced) which in effect could be salp muscles.

Finally, Harbison, Biggs & Madin (1977) found four Phronima *colletti* in barrels which "all had ridges resembling those on the test of *Salpa aspera*", and two females of *Phronima pacifica*, the smaller one in a barrel "fashioned from the posterior necto-

phore of the siphonophore, *Abylopsis tetragona*, while the barrel of the larger specimen resembled the test of *Salpa aspera*".

In spite of the century which has elapsed since the description of barrels first appeared in the scientific literature, their origin is not well documented, especially for the larger species, *Phronima sedentaria* and *P. atlantica*. While there is no doubt that the small species (*P. colletti*, *P. curvipes*, and *P. pacifica*) could make their houses from siphonophores or salps, the origin of those of *P. sedentaria* and *P. atlantica* is not clear.

Many *P. sedentaria* barrels do not look like pyrosomes (*e.g.*, compare Fig. 7 with the photograph of a Mediterranean specimen of *Pyrosoma* in Metcalf & Hopkins, 1919, Pl. 35, Fig. 48). Mediterranean pyrosomes caught in the area of this study are covered with numerous, contiguous very long test-processes; the tapering cormus is thick and perforated for insertion of ascidiozooids, the number of which is estimated to be about 800 for the part corresponding to the size of a barrel. On the other hand, amphipod houses have a barrel-like shape, with a rigid thin wall, adorned only with a few small tubercles more or less regularly disposed in parallel circles. If barrels originate from pyrosomes, the tunicate must undergo an elaborate change.

The great differences between barrels and pyrosomes led to a screening of many other potential hosts, rarer or more capable of avoiding nets: siphonophores, pseudothecosomate pteropods, heteropods, ctenophores, appendicularian houses, medusae, and even to consider a benthic origin, such as ascidians or anthozoarians. This search did not uncover any neglected animal, or part of animal, which could lead to the tuberculate barrel and it was then decided to undertake a methodical study of the barrels themselves.

A collection of barrels containing *P. sedentaria* (Forsk.) caught over many years were measured and scored for numerous qualitative characters. This paper will first present a multivariate analysis of these data leading to the recognition of several groups of similar barrels. Each group will then be examined in turn to find conclusive proof of its origin. The general scheme emerging from the study will be discussed.

Male *P. sedentaria* were not frequently caught with barrels, and it was decided not to include them in the morphometric analysis. A large number of barrels were collected during several years, but only the subset in which female *P. sedenturia* were observed alive, swimming with their barrels, before preservation in 5% formalin was used. Useful indications could thus be gained relative to the relationship between the amphipods and their houses, the number built by one female, correspondence between developmental stages and host species, and so on. These relationships will be the object of a subsequent paper.

MATERIALS AND METHODS

The total number of associations submitted to morphometric measurements was 70. Of this number, 47 were caught with a conical 1-m net (mesh-size 600 μm)

towed slowly (1 knot) at a depth of 50 m in the Bay of Villefranche (near Nice, Mediterranean Sea), mainly between 1963 and 1969. The small diameter of the net, and the slow, short period of towing (15 minutes) resulted usually in the catch of 0 or 1 (rarely 2) female P. *sedentaria.* The cod-end content, brought back alive to the laboratory, was observed within 1 h. When a female is seen swimming with a barrel of suitable size, and no other female or empty barrel is present in the catch, there is almost no doubt that the barrel was the one in which she lived before capture.

The other 23 associations were collected off Villefranche Bay with a 10-foot Isaacs-Kidd midwater trawl, towed horizontally for 30 min at 3 knots, at depths ranging from 10 to 600 m, mainly between 1970 and 1973. The cod-end content was immediately observed on board and the associations sorted and preserved. The probability of barrel interchanges during the tow between the 2 to 10 females usually present was considered negligible.

PRELIMINARY OBSERVATIONS AND CHOICE OF CHARACTERS FOR THE DATA ANALYSES

A preliminary examination of the barrels with a binocular microscope disclosed several kinds of barrels, as was already known from the literature. Only one of them was distinctly different from the others. This type shows seven longitudinal ridges, of which three are paired or furcated. It obviously corresponds with Delle Chiaje's *"Doliolum sulcatum".* The other kinds of barrels were not so distinct. Because of many intermediary forms, any classification strongly depended on which character was chosen. It was possible, however, to separate a further class of a few small barrels, gelatinous and devoid of ornamentation.

The remainder (more than half the collection) was composed of rigid and usually large houses. Some extreme types were well characterized. One was provided with a few circles of asymmetrical tubercles; a second one was covered with papillae or symmetrical tubercles; and a third showed a smooth surface, often adorned with small denticles. The first and second types correspond to Delle Chiaje's *D. papillosum,* the third to *D. mediterraneum;* most of the barrels, however, did not fall into either category. It was not possible to decide if they were intermediary forms, or whether consideration of other characteristics would indicate their connection.

The morphometric analysis was undertaken to clarify this point. At first it was thought that a data analysis of measurements alone could show separate clusters, due to differences in shape and size of the original hosts. When this failed it was decided to add a set of qualitative characters to the measurements.

Numerical analysis is viewed here essentially as a means of summarizing multidimensional data too complex to be understood by visual inspection; the interpretation of the results still relies mainly on biological judgement.

QUANTITATIVE MEASUREMENTS

Five variables were measured with a binocular microscope and an eyepiece micrometer (Fig. 1), the barrel being in a vial with 5% formalin in sea water.

L : overall length; when the ends are irregular, the longest measurement was taken as total length.

A : inner diameter of the large aperture.

D: largest inner diameter (midship-frame).

B : inner diameter of the small aperture.

E : distance from D to B.

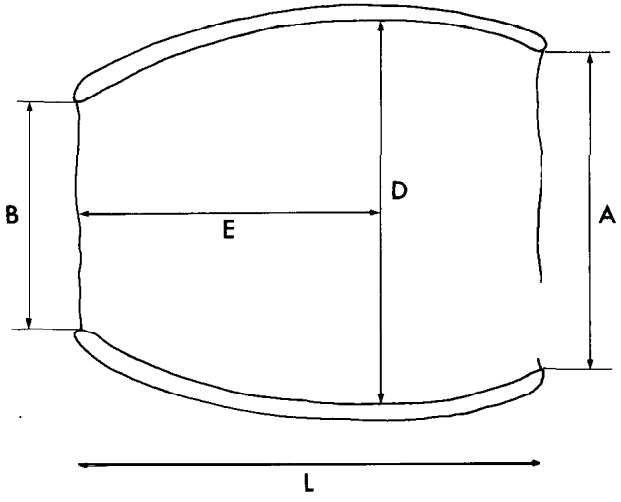

Fig. 1. Quantitative variables measured on the barrels: see text for identification of variables

When the diameters (A, D or B) were not completely circular, the mean of several measurements was taken. The shape of the barrels was never perfectly symmetrical, and the relative error was in the order of 1/20 (and even 1/10 for E). The thickness of the wall was not measured because it varies too much from place to place; it is correlated with the qualitative character 'test consistency'.

QUALITATIVE CHARACTERS

Most of the qualitative characters are best seen out of the preservative, owing to the different refractive index of the air. Staining with Methylene Blue, as advocated by Foxton (1965) for examination of salp tests, was tried but not found as effective as emersion to reveal minute details of ornamentation.

The six qualitative characters were coded in the following way.

T – Tubercles

O: absence – smooth surface, without protuberances; some scattered small denticles (0.05 mm) may be present.

1: corrugated surface, with no definite tubercles.
2: papillae or tubercles in a more or less alternate pattern, or a random pattern, close to each other.
3: asymmetrical tubercles, arranged in a small number of spaced rings (3 to 5, plus 1 incomplete ring at each end).

G – Granular aggregates
0: absence.
1: small condensations (covering no more than 0.2 mm in diameter) of fine particles included into the barrel wall; these aggregates arrange themselves in a more or less alternate pattern.

P – Polygonal network
0: absence.
1: ornamentation of very small (0.05 mm) denticles delineating polygons; often seen only at one end of the barrel.

C – Test consistency
1: very soft (gelatinous), not rigid in water.
2: collapses out of water.
3: keeps its shape out of water.

N – Annulations
0: absence.
1: presence of transverse constrictions.

R – Ridges
0: absence.
1: presence of longitudinal ridges, often one of which is furcated.

STATISTICAL ANALYSES

Uni- and bivariate statistics were calculated according to Sokal & Rohlf (1969) or Siegel (1956), and multivariate statistics mostly according to Cooley & Lohnes (1971). A principal component analysis was performed on the covariance matrix of quantitative characters. No logarithmic transformation was applied since size differences were of interest in this work.

To cope with both quantitative and qualitative attributes, Gower's general similarity coefficient was used (Gower, 1971):

$$S_{ij} = \sum_{k=1}^{v} s_{ijk} \Big/ \sum_{k=1}^{v} \delta_{ijk}$$

where S_{ij} is the similarity between individuals i and j, v the number of characters, and

$$\sum_{k=1}^{v} \delta_{ijk}$$

the number of characters over which a comparison is possible.

For dichotomous characters, s_{ijk} is 1 if both are present in observations *i* and *j*, and 0 otherwise; δ_{ijk} is 0 if both are absent, and 1 otherwise (*i.e.*, double absences are not considered a similarity).

For qualitative (multistate) characters, s_{ijk} is set to 1 if the two observations *i* and j agree in the kth character and to 0 if they differ.

For quantitative characters with values $x_1, x_2, \ldots x_n$ of character k for the total sample of *n* observations, $s_{ijk} = 1 - |x_i - x_j|/R_k$; R_k is the range of character k in the sample.

The ordination of the 70 x 70 matrix of similarity coefficients was carried out by principal coordinate analysis (Gower, 1966). A computer programme was written which followed, with numerous modifications, the PCOORD programme of Blackith & Reyment (1971). Subroutine HOW (Cooley & Lohnes, 1971) was used to factorize the matrix. The minimum spanning tree (MST) was computed and superimposed on the ordination. Single linkage clustering based on the MST (Gower & Ross, 1969) was also computed and visualized as a dendrogram.

No coefficients like "structure coefficients" (see Cooley & Lohnes, 1971, p. 106) are known for mixed data (quantitative +qualitative) in a principal coordinate analysis. To give some meaning to the axes, correlations between each variable and the principal coordinate scores on each axis were computed. For quantitative variables, this was done with the usual Pearson product–moment correlation coefficient. For qualitative variables the Spearman rank correlation coefficient was used, assuming an order, or at least an opposition, between the different states.

Programmes written in FORTRAN (principal coordinates, MST and single linkage clustering) were run on the IBM 7040 computer of the "Observatoire de Nice". All other statistical programmes were written in BASIC for a Hewlett-Packard 9830A desk-top computer, linked to a punched card reader and a plotter.

RESULTS

PRELIMINARY SCREENING OF THE QUANTITATIVE VARIABLES

Descriptive statistics for the five measurements are given in Table I. Coefficients of variation are always high, around 20%. Variances are of the same magnitude for all variables. The Kolmogorov–Smirnov test indicates that normality must be rejected at the 1% level for all variables except L (hypothesis intrinsic to the data, critical value according to Dagnelie, 1968). Every variable, except E, has a distribution skewed to the left (significant at the 5% level); in addition, the distribution of the largest diameter, D, is significantly leptokurtic. In fact, histograms (not shown here) give indications of bimodality for the three diameters, A, B and D.

Since no attempt to test cluster separation is intended, these deviations from normality are not important for the data analyses, which are only used to show the data structure in a space of reduced dimension.

TABLE I

Descriptive statistics for the 5 quantitative variables measured on the sample of 70 barrels: $\bar{X}$, mean (mm); $s_{\bar{x}}$, standard error of the mean; s^2, variance; CV, coefficient of variation; s_{cv}, standard error of CV; g_1, skewness; g_2, kurtosis; D, Kolmogorov–Smirnov statistic: see p. 191 and Fig. 1 for key to variables.

Variable	$\bar{X}$	$s_{\bar{x}}$	s^2	CV	s_{cv}	g_1	g_2	D
L	23.6	0.54	20.44	19.1	1.67	−0.8539	0.8036	0.0778
A	11.8	0.32	7.28	22.8	2.02	−0.9132	0.5376	0.1286
D	16.1	0.40	11.04	20.6	1.82	−1.1550	1.1854	0.1545
B	9.51	0.24	3,93	20.8	1.84	−0.6263	0.8018	0.7140
E	14.5	0.41	11.97	23.9.	2.14	−0.2120	0.6737	Q1629

PRINCIPAL COMPONENT ANALYSIS OF MEASUREMENTS

The first eigenvalue accounts for 89.6% of the total variance, and the corresponding axis is positively and highly correlated with the five variables (size axis). The second factor (6.8% of trace) compares longitudinal measurements (L and E) to transverse ones (A, D and B). The third axis (2.2% of trace) contrasts L with E and B, *i.e.*, large, inflated and symmetrical barrels with small, cylindrical and asymmetrical ones.

The resulting component scores are not given here, since they failed to show any distinct clusters. In the planes of axes 1–2, 1–3, and 2–3, the observations are scattered in a random fashion and, apart from a few extreme outlying values, the tentative groups which could be made in one plane do not stand out in the others.

This may be due either to the great variability present or to the fact that different hosts shaped by the amphipod reach the same interior sizes and proportions. While the latter hypothesis could be true (see Discussion), variability is in fact sufficiently high to cancel intergroup differences. For example barrels with ridges, which will be later designated as the 'A' group, show coefficients of variation of 20.1%, 15.1%, and 21.8% for the three diameters A, D, and B, respectively.

PRINCIPAL COORDINATE ANALYSIS OF QUANTITATIVE–QUALITATIVE CHARACTERS

The use of Gower's general similarity coefficient provides a means of combining size measurements with other non-metric characteristics. The first five axes account for 71.5% of the total variance. The percentages of trace explained by each factor are given in Table II, together with the correlations of the original variables with the resulting axes.

Only the graphs of axes 1–2 and 1–3 are given here (Figs 2, 3), because axes 4 and 5 introduce no new variables and are only weakly correlated with the measurements.

High values of measurements are expressed towards the positive pole of both axes 1 and 2, giving a size dimension to the first diagonal of the plane 1–2 ; hence this diagonal represents a time (growth) direction.

TABLE II

Correlations between the original variables and the resulting principal coordinate scores on the first 5 axes; upper part, Pearson product-moment coefficients for quantitative variables; middle part, Spearman rank correlations for qualitative variables; lower part, eigenvalues and proportions of total variance: see text pp. 191 and 192 for key to variables.

Variable	Factor				
	1	2	3	4	5
L	0.691	0.545	0.311	−0.061	0.107
A	0.656	0.549	0.334	−0.250	−0.033
D	0.685	0.534	0.378	−0.156	−0.014
B	0.588	0.535	0.311	−0.201	−0.065
E	0.642	0.480	0.315	−0.086	0.111
T	0.598	−0.366	0.289	0.340	−0.327
G	0.083	−0.528	0.734	0.303	−0.057
P	−0.199	−0.597	0.405	−0.463	−0.113
C	0.825	−0.004	−0.045	−0.018	0.167
N	−0.697	0.629	0.280	0.159	−0.105
R	−0.724	0.679	0.189	0.131	−0.037
Eigenvalue	9.7617	5.1846	3.1619	2.0666	1.6058
% trace	32.1	17.0	10.4	6.8	5.3

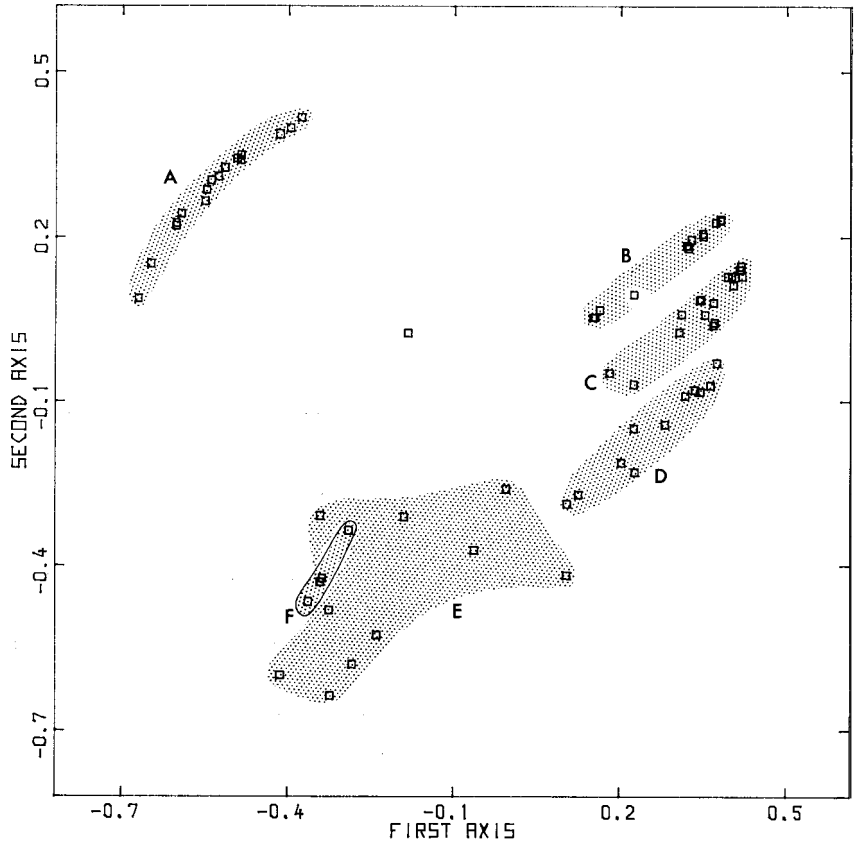

Fig. 2. Principal coordinate analysis of quantitative +qualitative characters of the barrels: axes 1 and 2; groups A to F were defined after pairwise examinations of axes 1 to 5.

Groups of scores may be formed by examination of successive planes combining the first 5 axes by pairs. Six groups were thus recognized, which behave consistently over all pairs. They are arbitrarily labelled clockwise from 'A' to 'F'. Only two barrels do not fit in any group. Judging only from the pattern of principal coordinate scores in planes 1–2 or 1–3 (Figs 2, 3), Groups 'B' and 'C', and possibly 'D', could be lumped together; their position on the remaining axes, however, precludes this condensation.

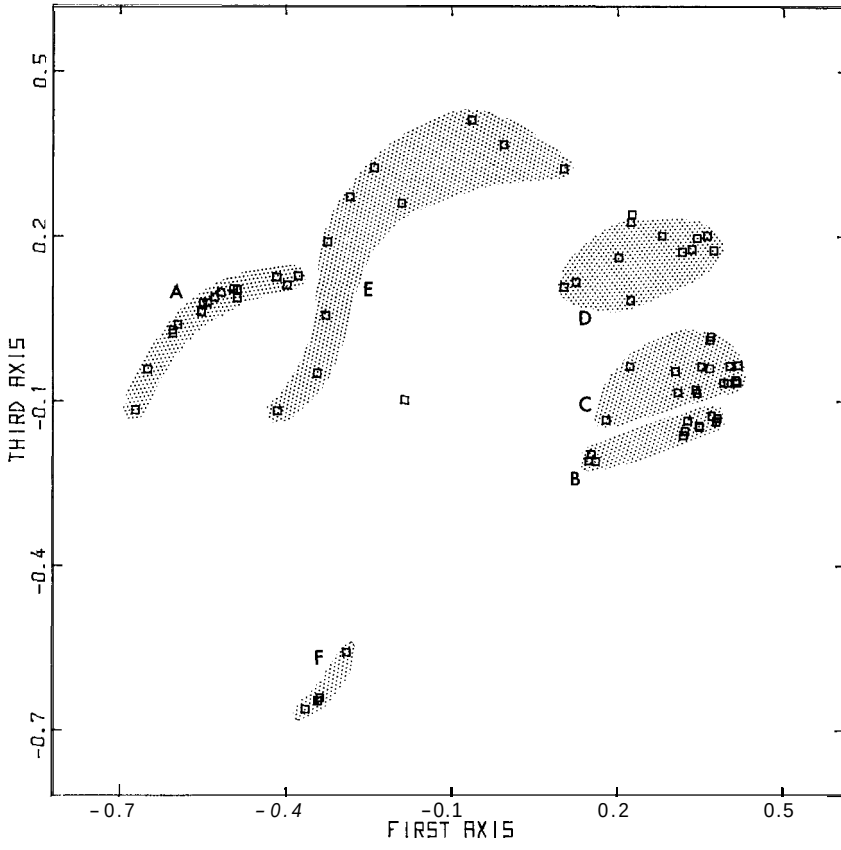

Fig. 3. Principal coordinate analysis of quantitative + qualitative characters of the barrels: axes 1 and 3.

The first three factors account for only 59.5% of the total variation. The dendrogram, which summarizes the similarities in the whole 11-dimensional space, shows that Groups 'A', 'B', and 'F' are well separated. The remaining Groups 'C', 'D', and 'E' are composed of several interrelated subgroups joined at a low level of similarity. This dendrogram is not given here, because its interpretation requires identification of individual observations. Instead the MST corresponding to the dendrogram

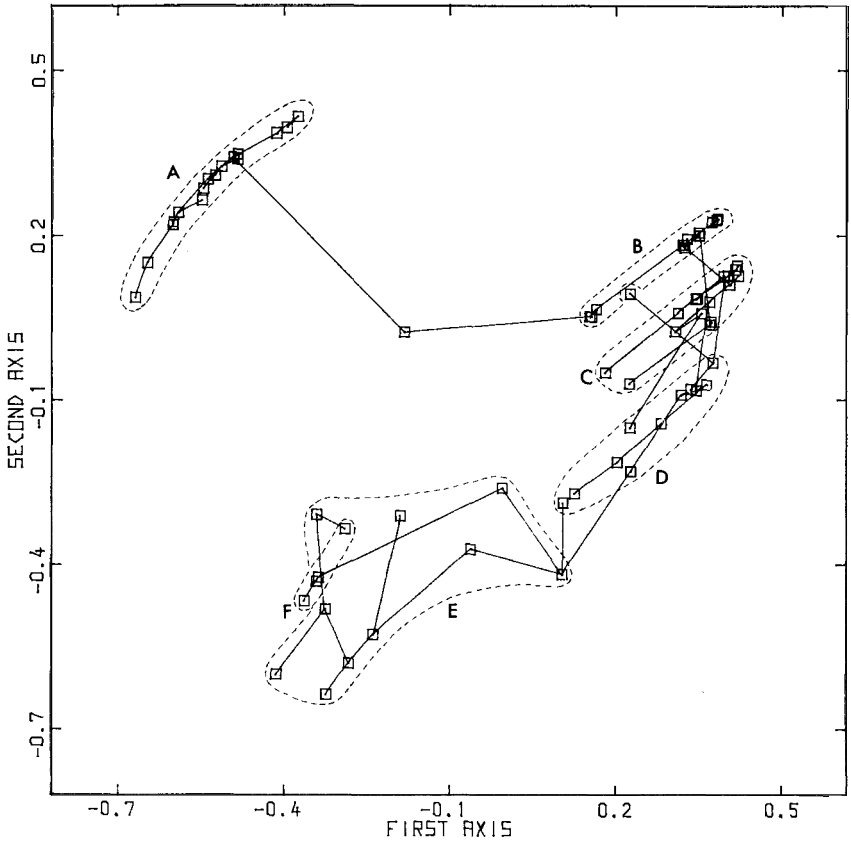

Fig. 4. Same as Fig. 2. with the minimum spanning tree superimposed on the graph.

(Gower & Ross, 1969) is superimposed upon the graph of factors 1 and 2 (Fig. 4). It indicates that 'A' is well isolated, 'B' has only two connections with 'C', and 'F' one with 'E'; 'E', 'D', and *'C'* are linked together.

BIOLOGICAL INTERPRETATION OF PRINCIPAL COORDINATE GROUPS OF SCORES

The six homogeneous groups of barrels detected by the multivariate analysis were submitted to a critical evaluation. They appeared to be classifiable in only two sets, from the point of view of the biological origin.

Groups derived from salps

Thr 'A'gvoup. All barrels with 7 longitudinal ridges and annulations (16 observations) fall into the 'A' group. The overall length, L, ranges from 18 to 27 mm, and the largest diameter, D, from 10.0 to 19.2 mm.

As stated before, this group was readily recognizable before the numerical analysis, owing to the peculiar and constant ornamentation of the test. Measurements and principal coordinate analysis further demonstrate that it is well defined, as shown by the elongated scatter of scores along the size direction (first diagonal).

The furcated ridge, which is the most distinctive feature of the ornamentation, was sought in all possible groups of gelatinous macroplankton. It was eventually found on the bulge surrounding the nucleus of the oozooid (solidary form) of Salpa *fusiformis* Cuvier. A thorough examination of the test of this form showed that all other ridges and grooves could be found on the barrels (Fig. 5); the annulations themselves represent the location of former muscles. There is, of course, a certain amount of test contraction following the removal of the "mantle" (in the terminology of Yount, 1954) by the amphipod, especially on the dorsal side of the salp, which is thinner and less rigid on the live animal. When the salp is swimming,

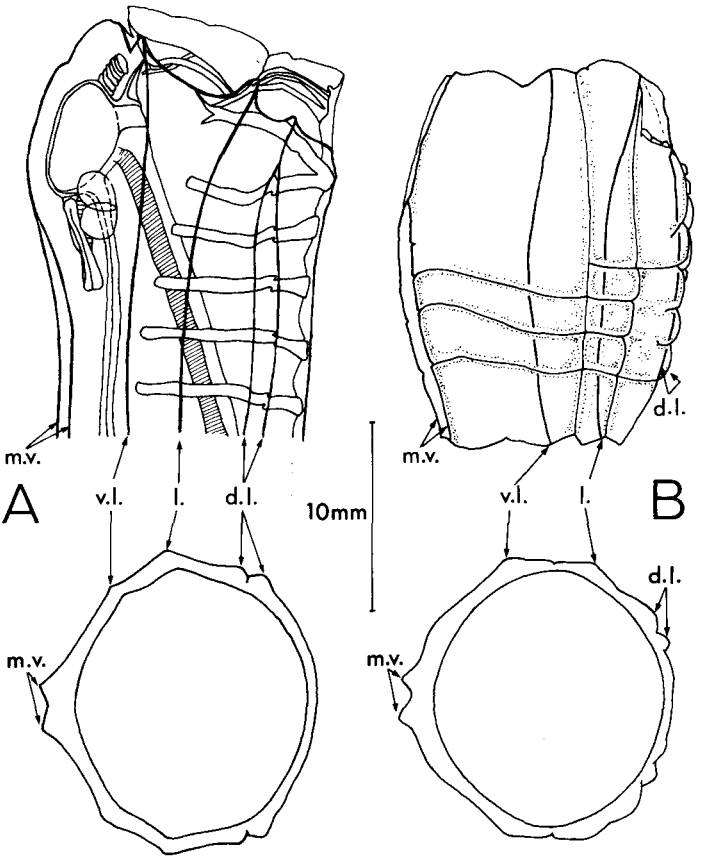

Fig. 5. Cornparison of the posterior region (right side) of an oozooid of Salpa *fusiformis* (A) with an 'A' group barrel (B): the system of longitudinal ridges is thickened; m.v., midventral ridge (furcated); v.l., ventrolateral ridge; l., lateral ridge; d.l., dorsal-limiting ridge (furcated).

this dorsal side is alternately inflated and depressed, and the classical term "dorsal longitudinal depression" (Stiasny, 1926; Yount, 1954) refers to the animal in preserved condition – generally a poor one. The opportunity was taken to examine some specimens of *S. fusiformis* preserved by Dr J.C. Braconnot, using a special method (Braconnot, 1973); they retained a life-like appearance and did not show any dorsal depression or the flattened and angular aspect of the section sketched by Stiasny (1926) and reproduced everywhere. Incidentally, it is not the ventrolateral ridge which lies across the muscles' extremities as shown by Stiasny (1926, Pl. 21a; Fig. 20), but the lateral one; the ventrolateral ridge stands between the latter and the furcated midventral ridge (see Fig. 5).

Apart from a few exceptional captures, only five species of salps occur in the area of the Mediterranean Sea sampled: *S. fusiformis*, *S. maxima* (Forsk.), *Thalia democratica* (Forsk.), *Pegea confoederata* (Forsk.), and *Ihlea punctata* (Forsk.). The last two species are less abundant. Both forms of *Thalia democratica* and the aggregate form (blastozooid) of *Salpa fusiformis* may be excluded as candidates for 'A' group barrels as their size is too small. *S. maxima,* whose internal anatomy is close to *S. fusiformis,* does not show the distinctive ridges of the test of the latter; the ridges also do not appear on the sexual and asexual forms of *Pegea confoederata* and *Ihlea punctata.*

The 'F'group. There are only four barrels in this well-defined group. They have in common a small size (range 9.9–18.7 mm for overall length and 5.6–10.8 mm for largest diameter) and a soft, transparent test devoid of any ornamentation. The barrel is almost invisible in the water. It is of course difficult to infer the origin of the host in absence of any peculiarities. Nevertheless, the appearance of the test is strongly reminiscent of a salp tunic in shape, thickness, glass-like transparency and outer surface of the wall.

During rearing experiments, two observations were made which could support the salp hypothesis. A male *Phronima sedentaria* caught at stage V (see Laval, 1975 for definition of stages), the first stage out of the mother's barrel, was given an oozooid of *Thalia democratica.* The oozooid length was 10.7 mm (without the long processes). Within 5 min, the male grasped it, cut a hole near the oral aperture and entered the salp. Ten minutes later the gill was eaten and the gut deeply injured; the male then left it and scraped the epidermis for 5 min, with the mouthparts, the head moving along the longitudinal axis of the salp.

The male then resumed eating the gut, and the nucleus completely disappeared in 4 min. Muscles and epidermis were afterwards scraped again. Then the male moved to the atrial aperture, enlarged it and, passing its abdomen through the hole, began to propel the salp in the typical manner of a phronimid moving a barrel. Subsequently, the only behaviour of the male was a slight scraping of the inner wall. Thus in less than 1 h only the test without any internal organs remained with the two apertures enlarged. Scraping of the wall continued intermittently for

several hours. Twelve hours later, the inner wall was perfectly smooth, the tunic was thicker (by contraction) and the apertures further enlarged. The test resembled a barrel, except for the two long posterior appendages of the zooid, which remained another day; they were then cut but not eaten by the amphipod. At this time the male moulted to stage VI.

The other observation is similar and concerns a juvenile female *Phronima sedentaria* which was given a *Salpa fusiformis* blastozooid. A circular opening was cut at the base of the long posterior 'horn' and the inside was eaten and scraped as in the preceding observation. A second opening at the base of the anterior process was made 5 h later. The posterior and anterior horns were separated 8 and 15 h, respectively after the beginning of the work, leading to a perfect barrel, well within the range of 'F' group barrels.

These observations show that small salps could be used by *Phronima sedentaria* and transformed to such an extent that nothing but the test (devoid of any organ, smoothed internally and open at both ends) remains visible. The specific identity of the salps used is difficult to determine because of the lack of any structure. In the area only oozooids and blastozooids of *Thalia democratica,* and blastozooids of *Salpa fusiformis* and *Ihlea punctata* are in the size range of 'F' group barrels.

Groups derived from pyrosomes

The 'E' group. The 10 barrels constituting this group are not so tightly clustered on the graphs (Figs 2, 3) as the other groups, indicating that it is more variable. The overall size is small, its range beginning at 14 mm (below the 'A' group) and ending at about 25 mm (largest diameter from 7.3 to 17.6 mm).

Every barrel in this group shows traces of a polygonal network. The outer wall is generally corrugated, with small elevations in a more or less alternate pattern; only two barrels can be described as smooth. Granular aggregates are present in all barrels, except for three small ones (on the extreme left of axis 1). Eight out of the 10 barrels are of medium consistency (coded 2), the remaining two are rigid (and large). An 'E' group barrel is shown in Fig. 6; it is somewhat exceptional in the extension of the polygonal ornamentation, which covers all the surface; normally the network is only visible at a few places, usually around the small aperture.

The discovery of a barrel of smaller size, with an outer surface formed of polygonal facets, was a clue for the identification of the origin of the 'E' group (this barrel was not included in the numerical analysis because it was not caught with an amphipod). These facets constitute the ornamentation of colonies of young *Pyrosoma* and when one of them was placed by a barrel, the similarity was striking. The polygonal facets in *Pyrosoma* disappear when elevations, and later protuberances. grow at the surface of the colony. The closed extremity of the cormus being older, it is not surprising to find remains of polygonal lines on the small aperture of barrels.

The granular aggregates present in the wall of the barrels are easy to explain

in the light of a pyrosomian origin. They do not come from the withdrawn zooids, which are too numerous and crowded (Fig. 8), and may be completely extracted by the amphipod – their epidermis being attached to the test only at the oral and atrial apertures (Metcalf & Hopkins, 1919, p. 202). They could only arise from decay

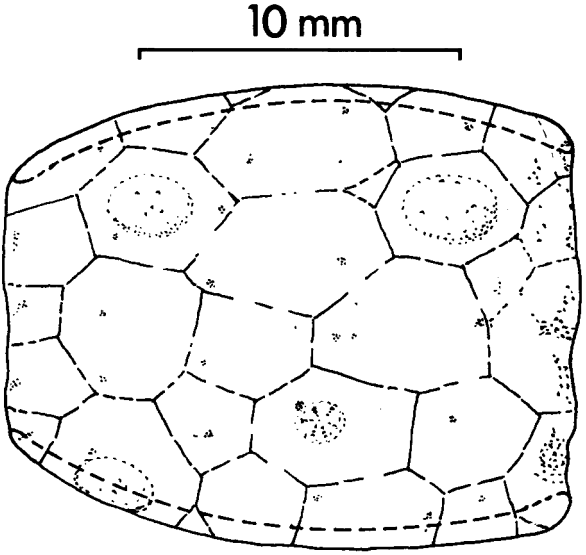

Fig. 6. An 'E' group barrel with polygonal network of very small denticles extending over the whole test surface.

of the secondary blastozooids produced by budding of the pre-existing zooids; when not fully grown, these buds make spheres of living tissues included in the test wall and fed by the stolon; when the zooids are removed by the amphipod, these spheres thus remain in the wall and begin to decompose.

The presence of elevations on the outer wall in an alternate pattern also agrees with an origin from young colonies of Pyrosoma.

The 'D'group. This group of 11 barrels is more precisely defined than the 'E' group on the plane of axes 1 and 2 (Fig. 2), where it can readily be seen that it extends the latter in the 'size direction' (first diagonal). This is confirmed by the measurements: overall lengths range from 21 to 29 mm and largest diameters from 13.0 to 19.9 mm.

Apart from the larger size, barrels of the 'D' group are distinguished from the 'E' group by the lack of polygonal lines in all but two observations, test consistency which is always coded as rigid, and protrusions on the outer surface usually scored 2 (papillae) or 3 (tubercles). Granular aggregates are present in all barrels, except in the two already singled out by the remains of polygonal network. These barrels lie near the startingpoint of the 'D' group in the size direction and constitute a transition to the 'E' group.

According to the explanation in the section above, the presence of granular aggregates qualifies the 'D' group for a pyrosomian origin. The larger size relative to the 'E' group is sufficient to explain the disappearance of the polygonal network, the higher rigidity of the test and the apparition of papillae and tubercles.

The 'C'group. This group (16 barrels) is not very different from the 'D' group. Overall sizes are similar (range, 20 to 31 mm). Largest diameters (which range from 15.4 to 20.6 mm) seem slightly larger in the 'C' group. The difference was found significant at the 5% level by the Mann-Whitney U-test.

The main difference between the two groups is the lack of granular aggregates, which is primarily responsible for the separation of groups 'D' and 'C' on the second axis (Fig. 2). Absence of granular aggregates is not a positive character ; they may well have been unnoticed. In fact, a thorough re-examination of the 'C' group barrels with a higher magnification showed, in some houses, granular traces arranged in the same alternate pattern. The distinctness of the two groups is thus somewhat artificial; 'C' group barrels are only 'D' group barrels in which granular aggregates are not visible. They could come from a part of a pyrosome cormus devoid of young secondary blastozooids, or might be older 'D' barrels in which decaying particles have had time to disappear.

There are more barrels coded 3 for the ornamentation in the 'C' group (8 out of 16) than in the 'D' group (3 out of 11). A 'C' group barrel with parallel rings of tubercles

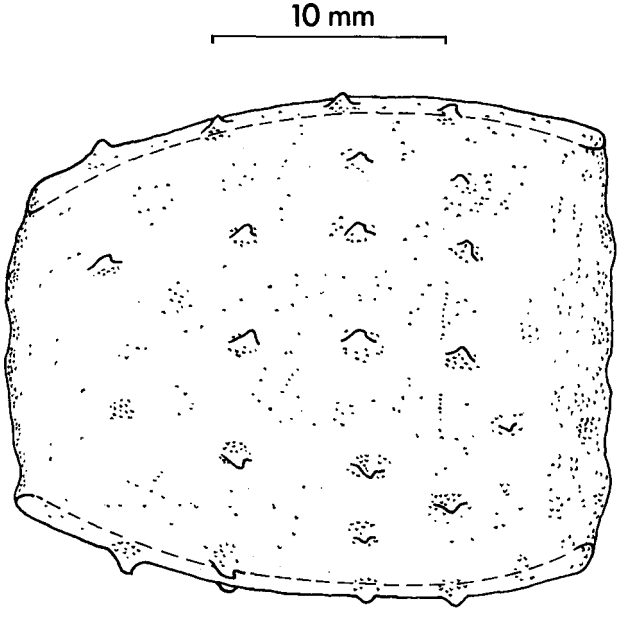

Fig. 7. A 'C' group barrel with tubercles: stippling depicts small denticles; granular aggregates are present but too small to be seen.

(=code 3) is shown on Fig. 7, but it should be stressed that the 'C' group (as well as the 'D' group) contains barrels coded 1, 2 and 3 for ornamentation.

A decisive advantage of the multivariate analysis is apparent at this point. Prior to the analysis, barrels with parallel and spaced rings of tubercles (such as the one of Fig. 7) were thought to represent a peculiar category; their pyrosomian origin was strongly questioned as too far from the characteristic aspect of colonies of Mediterranean *Pyrosoma* (see p. *189)*. The analysis clearly demonstrates that barrels coded 3 for ornamentation do not constitute a separate cluster. They are not even different in maximum diameter from barrels coded 2, as shown by a Mann-Whitney U-test (11 barrels coded 2 versus 11 barrels coded 3, from 'D' + 'C' groups; $U = 51$ while $U_{0\,05} = 34$ – one-tailed test: '3' > '2').

Origin of the tubercles. Three barrels from the 'D' groups possess both granular aggregates and small tubercles, and the tubercles found on eight barrels of the 'C' group do not differ from those of the 'D' group. How then can one explain this formation of small asymmetrical tubercles from the long finger-like processes (Fig. 8) of pyrosomes? Some observations on living animals may help to clarify this. Five associations of female *Phronima sedentaria* with pyrosomes were caught in plankton nets on different occasions and brought back alive to the laboratory. In each association the amphipod was inside the pyrosome. Colonies were of small to medium size, and three of them were still closed at the apex. Survival was not good because of inadequate conditions of rearing, and observations could not be pursued after a few days. At that time, the colony always looked like a cylindrical tube open at both ends. It was not very rigid, and was different from the shape and consistency of a true barrel.

It is difficult to say whether the female was engaged in the process of making a barrel and had been disturbed by bad conditions of rearing, or was only eating a pyrosome with the same stereotyped behaviour used in the making of a house. In any event, these observations give indications on the reaction of the pyrosome when eaten by the amphipod. The female held on the inner wall with its pereopods, and scraped the test of the colony with its mouthparts (which were cleared frequently by the gnathopods). When a zooid was encountered, it was bitten and extracted by a sudden backward motion of the head. The zooid was then eaten with the aid of the gnathopods; when the amphipod stomach is full, the zooids are frequently ignored by the female. It is interesting to note that, following zooid extraction, the long papilla strongly retracts, and the cavity itself collapses. About 10 h later, the remaining canal is filled with a granular, soft, sticky substance, overflowing to some extent onto the outer surface of the test.

When stained with aceto-glucosic carmine and examined with a microscope, this material shows a network of living cells with ramified cytoplasmic expansions, a big nucleus, and numerous vacuoles embedded in a transparent substance. These cells are obviously analogous to the large tunic cells ("grandes cellules

tunicières") in the tunic of colonial ascidians described by Pérès (1948a), which are able to gradually transform their cytoplasm into tunical substance. In non-colonial ascidians, ablation of a fragment or of the whole tunic induces a regeneration from the ectodermis (Fol, 1908; Pérès, 1948b). In the case of zooid extraction from the common tunic of a colony of *Pyrosoma*, the ectodermis is withdrawn with the zooid. It is possible that migration of tunic cells occurs to fill the space with new material.

The barrel wall could thus be formed from a pyrosome cormus if the long papillae (Fig. 8) were retracted and the cavities formerly occupied by the zooids were re-filled with material secreted by the cells of the tunic. Disappearance of small protrusions

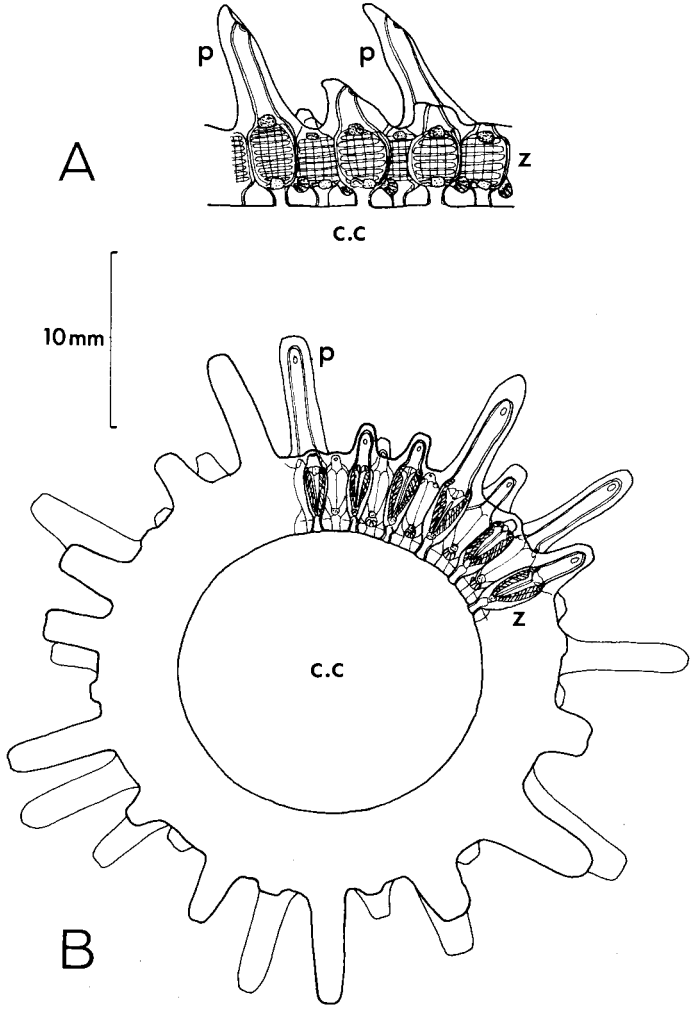

Fig. 8. Thick sections of a colony of *Pyrosoma atlanticum*. A, longitudinal section of the wall; B, cross-section of the cormus; p, long hollow papilla; z, blastozooid; c.c., common cloacal cavity.

would leave a smooth surface; incomplete disappearance of large protrusions would leave a corrugated or papillose outer surface. Some colonies of *Pyrosoma* have been found where the long finger-like processes did not occur on the entire length of the cormus, but only on every fourth ring of zooids; the other zooids were almost without processes. Perhaps there are different lines of descent among the blastozooids. This could explain barrels like the one in Fig. 7.

During the period of zooid extraction, the female scraped the inner surface, and continued to rub it when extraction was complete. The spherical small secondary blastozooids could then be seen imbedded in the wall, as suggested for the origin of granular aggregates.

The 'B' group. In their measurements, the 11 'B' group barrels come very close to the 'C' group; overall lengths range from 22 to 31 mm and maximum diameters from 16.0 to 20.8 mm. They are homogenous in test consistency (firm), and are all devoid of polygonal lines and granular aggregates. The outer surface is always smooth and covered with short lines (0.4 to 2 mm) of very fine (0.05 mm) denticles; the orientation of the lines changes from place to place. A typical 'B' group barrel is shown in Fig. 9; the test is generally thicker than in the other groups.

The origin of 'B' group barrels was difficult to trace. Their smooth outer surface and their large size seemed to rule out the possibility of a pyrosomian origin. Presence of a bulge on the wall of three barrels suggested a salp as precursor, as

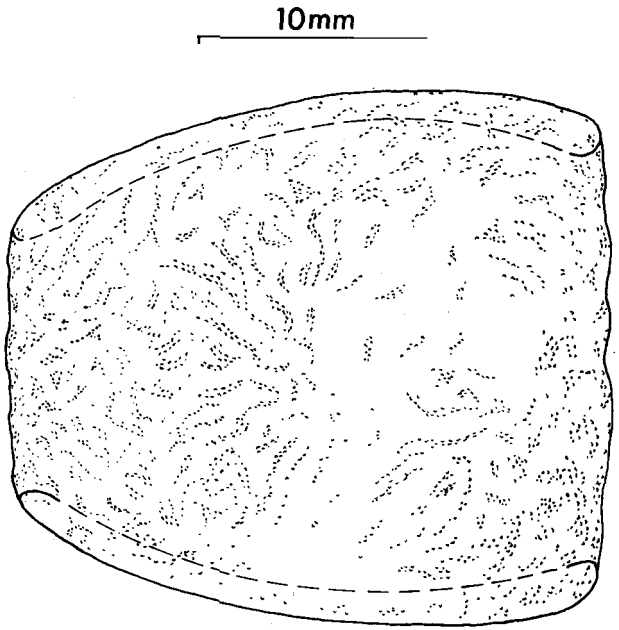

Fig. 9. A typical 'B' group barrel, with its ornamentation of very small denticles.

many species of them possess a swelling over the nucleus. The tunic of every species of salp likely to be candidate (see 'A' group) was thoroughly examined for presence of the ornamentation of denticles. Results were completely negative. Moreover, except for *Salpa fusiformis* oozooids which constitute the 'A' group, the test of salps, except for the nuclear swelling, was found to be too soft for an acceptable barrel. Returning to the barrels, the following facts were established. First, numerous denticles are present on the *inner* surface of the wall. This excludes salps as candidates, because the inner test surface is coalescent with the ectodermis in a salp; on the other hand, the surface facing the lumen of the cormus is still an external layer in a pyrosome. Secondly, test processes of colonies of *Pyrosoma* are adorned with longitudinal lines of denticles, which agrees closely with the barrel denticles; denticles with the same aspect are also found on 'D' and 'C' group barrels. If one admits that test processes collapse after zooid extraction, one could have an explanation for the pattern of lines of denticles found on 'B' group barrels.

It should be pointed out that presence of denticles on the inner wall is not compatible with a scraping of the test; this agrees with the greater thickness of 'B' group walls. One reason for the lack of scraping of the test could be absence of secondary blastozooids which do not need to be removed to prevent decomposition. Thus, a probable hypothesis for the origin of 'B' group barrels is: a section of a large *Pyrosoma* cormus with small finger-like processes (allowing complete retraction) and without young secondary blastozooids.

The two unclassified barrels

The barrel which can be seen in the centre of Fig. 2 is, by its ornamentation, a small 'B' barrel which was less rigid and thus coded 2 instead of 3 for test consistency. This is sufficient to explain the shift to the left on axis 1 (highly correlated with size and consistency). The 'B' group membership is confirmed by presence of some small denticles on the inner test surface.

The other isolated barrel (projecting onto the 'B' group in Fig. 2 and onto the 'D' group in Fig. 3) is a 'D' group barrel by all its characters, with transverse grooves close to the circles of tubercles. Its pyrosomian origin is ascertained by a conspicuous alternate arrangement of granular aggregates. The grooves are manifestly hazards of tunical growth.

DISCUSSION

Data analysis of *Phronima sedentavia* barrels, followed by a close examination of the different classes resulting, thus uncovers only two macroplanktonic hosts for origin: salps and pyrosomes. This parentage has been postulated by previous workers, but was not based on firm evidence.

Small salps difficult to identify with certainty *(Thalia democratica,* or the ag-

gregated forms of *Salpa fuszformis* or *Ihlea punctata)* are used by juvenile *Phronima* upon leaving the materna1 house. The small numerical importance of this kind of barrel found in the present study should not be misleading. Their small size allows them to escape through meshes of pelagic nets, and they are likely to go unnoticed in most samples due to their perfect transparency and gelatinous, soft texture.

The largest salp barrels (from about 10 mm in diameter) all originate from the oozooid of *Salpa fusiformis*. This is established without question by the peculiar test ornamentation. Other salps apparently cannot be formed into an acceptable barrel. This statement is, of course, only valid for the area studied (Ligurian Sea), as are other host origins given in this paper; *Phronima sedentaria* has a circumglobal geographical distribution (Shih, 1969) and other hosts could well replace the Mediterranean ones in other parts of the range.

All the other groups, which are indeed close together in the graphs, were eventually attributed to pyrosomes. This was, at first, difficult to visualize in some cases and raises the problem of the transformation of an animal into a barrel.

It was shown that all the tissues were carefully removed by the amphipod (in salps as in pyrosomes), leaving only the tunic. In pyrosomes, cavities left by the extracted zooids collapse and are filled by a new tunic issued from the special cells embedded in nearby areas. Long hollow finger-like processes retract to a degree depending on their initial size, leaving either a smooth, corrugated, papillose or tuberculate outer surface. The inner surface is generally scraped off, perhaps to eliminate decomposing secondary ascidiozooids remaining in the test after zooid extraction.

The inner surface is extremely smooth, as though finished with a fine cutting tool on a lathe. This could explain, besides the high variability, why principal component analysis did not disclose distinct groups of barrels. The inside is always made hollower in the middle, so that the wall is thicker at both ends. Less rigidity in the middle could produce the swelling leading to the barrel-like shape, but there could be also a mechanical distention by the rotating amphipod before tunic consolidation.

The barrel wall is stiffer than is the test of any of the host animals. This stiffening is either due to natural inflation of tunical substance after zooid or tissue removal, or induced by the amphipod. It should be stressed that *Phronima* possesses tegumental glands in the pereopods and mesosome. They are described by Mayer (1879) and Claus (1879). Mayer (1879) postulated a dissolving action of the secretion, to hollow out the barrel, but this was strongly disputed by Vosseler (1901). These glands are obviously analogous to the "cement glands" of gammaridean amphipods, and anyone looking at Fig. 2 in Goodhart's paper (1939) showing *Leptocheirus pilosus* (Photidae) in its tube should be impressed by the similarity with *Phronima* in its barrel. The same characteristic somersault movement of *Phronima*, fully described by Minkiewicz (1909), is observed in *Leptocheirus* when reversing its orienta-

tion in the tube. While these remarks are of prime importance from a phyletic point of view, they do not lead to an understanding of the rôle of these glands. In gammarids the sticky secretion of the glands is used to aggregate detritical particles for building the tube wall; it does not remain sticky but hardens. In *Phronima sedentaria* the glands are conspicuous and have ducts opening at the pereopod extremities. For the present, the tentative suggestion is made that they may be used for preserving or hardening the tunic, perhaps by a kind of 'tanning' action; I hope to be able to clarify this question later.

If the largest barrels ('C' and 'B' groups) come from pyrosomes, they must originate from rather large colonies. A sample of 22 colonies of *Pyrosoma*, chosen to illustrate the size range caught in the area, was used to show the length–diameter relationships. Thick sections were cut at a distance of four zooids rows, from both extremities of the cormus, and the inner diameters measured (with a precision not better than 1/15). The relationship with the colonies overall length is shown in Fig. 10. Owing

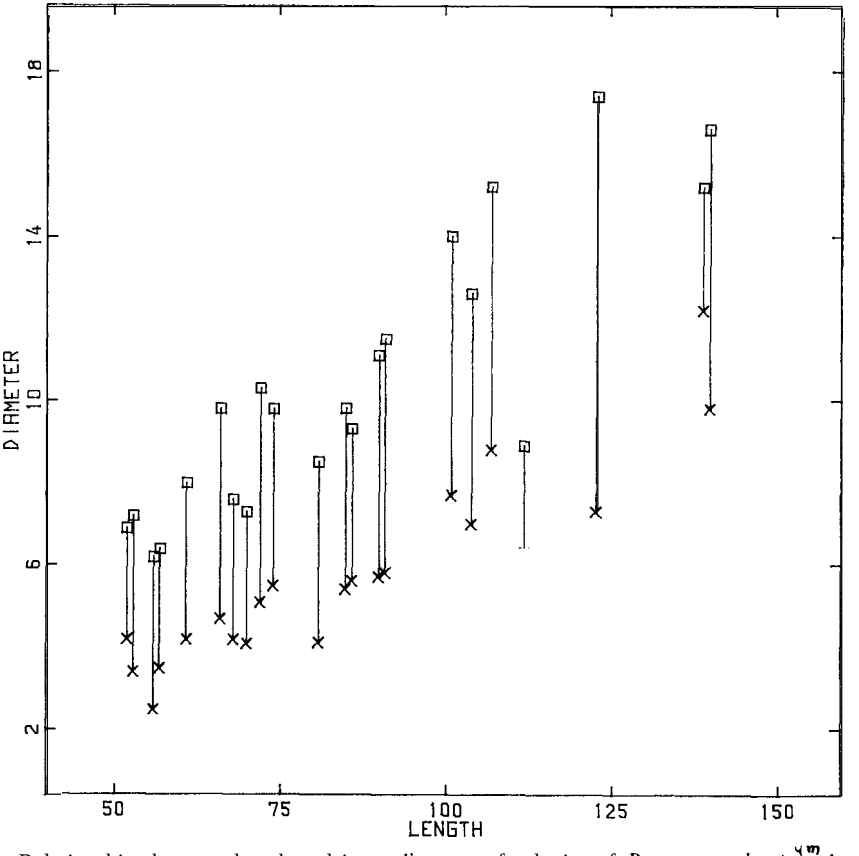

Fig. 10. Relationships between length and inner diameter of colonies of *Pyrosoma atlanticum*: the diameters (mm) of both the open (□) and closed (x) ends of the colonies were measured; the values for each colony are joined by a line.

to the greater hollowing in the middle of barrels, it is better to consider the barrel's large aperture for comparison. Minimum, mean, and maximum inner diameters are 11.7, 14.2, and 15.8 mm, respectively, for the 'B' group large apertures (corresponding measurements for the 'C' group are a little less). Considering that barrel diameters are surely increased due to scraping, this is well within the range of available *Pyrosoma* colonies.

An important variability is apparent for cormus proportions in Fig. 10; it is already obvious by simple visual inspection. Squat, elongated, tapering or near-cylindrical colonies all exist in plankton. It is, therefore, very difficult to decide from what part of a colony a particular barrel originates.

Some information about size distribution of *Pyrosoma* colonies in plankton (of which there is little in the literature) has been provided by Dr Braconnot (unpubl. data). Over a 3-year period, 175 tows were made in the area with a 10-foot Isaacs-Kidd midwater trawl, at depths ranging from 50 to 1500 m. A total of 6117 *Pyrosoma* colonies (all referred to *Pyrosoma atlanticum* (Péron)) were counted and measured, of which 5551 were under 30 mm long. Only 15 had an overall length between 8 and 14 cm. Thus, colonies corresponding to 'B' and 'C' groups are not frequent. But it should be remembered that *Phronima* eyes are perfectly adapted for high sensitivity (Ball, 1977) and perhaps should be able to see a luminescent pyrosome at some distance.

Finally, the relationship between *Phronima* and its barrel deserves some consideration. It is not in essence predaceous, because salps or pyrosomes are not primarily used as food (although they may be, besides making a barrel); moreover, the amphipod remains with – within – its partner. It is rather a borderline case of parasitism, soon fatal to the host, which is killed to obtain advantages from its tunic. Some hermit crabs are known which similarly kill and eat molluscs before occupying the shells (Rutherford, 1977). Most of the hyperiid amphipods (if not all, as postulated by Harbison *et al.*, 1977) are parasitic on pelagic animals. Phronimids, which deposit their larvae in the barrel, as do many hyperiids on their hosts (Laval, 1965), are not exceptions to the rule. By killing their host and removing its soft parts to prevent decay, they only go a step further and thus approach the margins of predation.

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This paper is dedicated to Professor P. Drach as a (belated) homage for his scientific jubilee.

REFERENCES

BALL, E. E., 1977. Fine structure of the compound eyes of the midwater amphipod *Phronima* in relation to behavior and habitat. *Tiss. Cell*, Vol. 9, pp. 521–536.

BLACKITH, R. E. & R.A. REYMENT, 1971. *Multivariate morphometrics.* Academic Press, London & New York, 412 pp.

BRACONNOT, J. C., 1973. Contribution a l'étude des stades successifs dans le cycle des Tuniciers pélagiques Salpides en Méditerranée. *Bull. Inst. océanogr., Monaco,* Vol. 71, Bull. No. 1424, 27 pp.

CHUN, C., 1889. Bericht über eine nach den Canarischen Inseln im Winter 1887/88 ausgeführte Reise, II. *Sber. preuss. Akad. Wiss.*, Bd 30, S. 519–552.

CHUN, C., 1895. Atlantis. Biologische Studien über pelagische Organismen, IV. Die Secundaren Geschlechtscharaktere der Mannchen von *Phronima. Biblthca zool.,* Bd 19, S. 107–130.

CLAUS, C., 1862. Bemerkungen über *Phronima sedentaria* Forsk., und *elongata* n.sp. Z. *wiss. Zool.,* Bd 12, S. 189–196.

CLAUS, C., 1872. Zur Naturgeschichte der *Phronima sedentaria* Forsk. *Z. wiss. Zool.,* Bd 22, S. 331–338.

CLAUS, C., 1879. Der Organismus der Phronimiden. *Arb. zool. Inst. Univ. Wien,* Bd 2, S. 59–88.

COOLEY, W. W. & P. R. LOHNES, 1971. *Multivariate data analysis.* J. Wiley & Sons, New York, 364 pp.

DAGNELIE, P., 1968. A propos de l'emploi du test de Kolmogorov-Smirnov comme test de normalité. *Biométr.-Praxim.*, T. 9, pp. 3–13.

DELLE CHIAJE, 1841. Descrizione e notomia degli animali invertebrati della Sicilia citeriore, Vol. 6/7, Napoli.

DUDICH, E., 1926. Systematische und biologische studien an den *Phronima*-Arten des Golfes von Neapel. *Zool. Anz.,* Bd 65, S. 117–139.

FOL, A., 1908. Note sur la régénération de la tunique chez les Tuniciers. *Bull. Soc. zool. Fr.,* T. 33, pp. 79–82.

FOXTON, P., 1965. An aid to the detailed examination of salps (Tunicata: Salpidae). *J. mar. biol. Ass. U.K.*, Vol. 45, pp. 679–681.

FRASER, J., 1962. *Nature adrift. The story of marine plankton.* G. T. Foulis & Co., London, 178 pp.

GOODHART, C. B., 1939. Notes on the bionomics of the tube-building amphipod, *Leptocheirus pilosus* Zaddach. *J. mar. biol. Ass. U.K.*, Vol. 23, pp. 311–325.

GOWER, J. C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika,* Vol. 53, pp. 325–338.

GOWER, J. C., 1971. A general coefficient of similarity and some of its properties. *Biometrics,* Vol. 27, pp. 857–874.

GOWER, J. C. & G. J. S. ROSS, 1969. Minimum spanning trees and single linkage cluster analysis. *Appl. Statist.*, Vol. 18, pp. 54–64.

HARBISON, G. R., D. C. BIGGS & L. P. MADIN, 1977. The associations of Amphipoda Hyperiidea with gelatinous zooplankton. II. Associations with Cnidaria, Ctenophora and Radiolaria. *Deep-Sea Res.,* Vol. 24, pp. 465–488.

LAVAL, Ph., 1965. Présence d'une période larvaire au début du développement de certains Hypérides parasites (Crustacés Amphipodes). *C. r. hebd. Séanc. Acad. Sci., Paris, T.* 260, pp. 6195–6198.

LAVAL, Ph., 1968. Observations sur la biologie de *Phronima curvipes* Voss. (Amphipode Hypéride) et description du mâle adulte. *Cah. Biol. mar., T.* 9, pp. 347–362.

LAVAL, Ph., 1975. Une analyse multivariable du développement au laboratoire de *Phronima sedentaria* (Forsk.), Amphipode Hypéride. Étude de l'influence de la température et de la quantité de nourriture. *Annls Inst. océanogr., Paris, T.* 51, pp. 5–41.

LEGENDRE, R., 1940. La faune pélagique de l'Atlantique au large du Golfe de Gascogne recueillie dans des estomacs de Germons. *Annls Inst. océanogr., Paris,* T. 20, pp. 127–310.

MAYER, P., 1879. Carcinologische Mittheilungen. *Mitt. zool. Stn Neapel,* Bd 1, S. 40–48.

METCALF, M. M. & H. T. HOPKINS, 1919. *Pyrosoma.* A taxonomic study based upon the collections of the United States Bureau of Fisheries and the United States National Museum. *Bull. U.S. natn. Mus.,* No. 100, Vol. 2, pp. 195–275.

MINKIEWICZ, R., 1909. Mémoire sur la biologie du Tonnelier de mer *(Phronima sedentaria* Forsk.). II: Le comportement: mouvements et réflexes, (étude expérimentale et opératoire). *Bull. Inst. océanogr., Monaco,* T. 152, pp. 1–19.

PAGENSTECHER, H.A., 1861. *Phronima sedentaria.* Ein Beitrag zur Anatomie und Physiologie dieses Krebses. *Arch. Naturgesch.,* Bd 27, S. 15–41.

PÉRÈS, J. M., 1948a. Recherches sur le sang et la tunique commune des Ascidies composées. I. Aplousobranchiata (Polyclinidae et Didemnidae). *Annls Inst. océanogr., Paris, T.* 23, pp. 345–373.

PÉRÈS, J. M., 1948b. Recherches sur la genèse et la régénération de la tunique chez *Ciona intestinalis* L. *Bull. Inst. océanogr., Monaco,* No. 936, 12 pp.

RUTHERFORD, J.C., 1977. Removal of living snails from their shells by a hermit crab. *Veliger*, Vol. 19, pp. 438–439.

SHIH, C.T., 1969. The systematics and biology of the family Phronimidae (Crustacea: Amphipoda). *Dana. Rep.,* Vol. 74, pp. 1–100.

SIEGEL, S., 1956. *Nonparametric statistics for the behavioral sciences.* McGraw-Hill, New York, 312 pp.

SOKAL, R.R. & F.J. ROHLF, 1969. *Biometry. The principles and practice of statistics in biological research.* Freeman & Co., San Francisco, 776 pp.

STIASNY, G., 1926. Ueber die Testa der Salpen und ihre systematische Bedeuntung. *Pubhl. Staz. zool. Napoli*, Vol. 7, pp. 385–457.

VOSSELER, J., 1901. Die Amphipoden der Plankton-Expedition. I Theil: Hyperiidea 1. *Ergebn. Atlant. Ozean Planktonexped. Humboldt-Stift.* Bd 2, G.e., S. 1–129.

YOUNT, J. L., 1954. The taxonomy of the Salpidae (Tunicata) of the central Pacific Ocean. *Pacif. Sci.*, Vol. 8, pp. 276–330.