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Brachiopods in Mud: Resolution of a Dilemma

Abstract. *Assumptions made from studies of sparse living faunas of brachiopods, namely, that they are intolerant of mud, that the free-lying habit is confined to species without pedicles, and that the pedicle of articulate brachiopods is uniform in structure and function, do not withstand critical examination. Studies in New Zealand show that some species in the same area occur in both attached and free-lying populations. Individuals cannot always be differentiated morphologically, but the structure of populations from hard and soft substrates is distinctive. Attachment to a substrate appears to be a larval rather than an adult requirement in most species.*

When Thayer (*1*) asked why brachiopods no longer inhabit mud, he raised the dilemma encountered by all students of brachiopods. All living articulates are assumed to be confined to hard substrates and to be immobile suspension feeders. Their abundance in the originally soft sediments of Paleozoic deposits is thus inexplicable in terms of their known distribution in Recent seas and of the supposed requirements of suspension feeding animals. Another conundrum is the temporal distribution of attached and free-lying taxa. In the Paleozoic, pedicle and free-lying taxa were almost equally represented (*1*). In Recent seas only one species, *Neothyris lenticularis*, is assumed, from the morphological characters of dredged samples, to be free-lying on coarse sand and gravel substrates (*2*).

Apparent differences in the habit and habitat of Paleozoic and living faunas are reconciled by data from populations investigated in southern New Zealand. In these coastal waters the brachiopods, like their Paleozoic counterparts, are the dominant component of subtidal faunas. Nine species (representing three of the five orders of living brachiopods) occur in ranges of habitats in neighboring but different geological areas. All of these areas were accessible to scuba divers, thus allowing direct sampling and comparative studies of behavior, morphology, distribution, and population structures.

Because of the rarity and relative inaccessibility of brachiopods in modern seas, correlations between structure and life-style have had to be made from dredge hauls. Thus the characters of differential thickening and small foramen and beak of *N. lenticularis* have been equated with a free-lying existence (*3*), and the absence of thickening and the possession of a large foramen have been associated with a life of permanent attachment to a hard substrate. These assumptions have also been used to interpret fossil collections (*4*).

In coastal waters of southern New Zealand, species with characters previously associated with either attachment or free life are found to have both attached and free-lying populations, some of the latter in mud. Two areas have been studied in detail. (i) Long Sound in Preservation Inlet is a fjord with near-vertical granite walls and has a maximum depth of 380 m. The bottom sediments are anaerobic muds and currents are negligible in the areas sampled. The only available attachment surfaces are the rock walls. (ii) Paterson Inlet is a drowned river valley with a surface area of 65 km^2. It does not exceed depths of 45 m and provides a variety of habitats. The northern shoreline is largely granite boulders and outcrops, and numerous islands scattered throughout the inlet have rock surfaces. Sediments of shell gravel and sand surround the islands, particularly at the inlet entrance, but most of the floor is mud.

Four species of brachiopods are common to these two areas: *N. lenticularis*, *Terebratella sanguinea*, *T. inconspicua*, and *Notosaria nigricans*. All are attached to subtidal vertical rock walls in Long Sound (Fig. 1). In Paterson Inlet, individuals of *N. nigricans* are found only attached, adult *Neothyris lenticularis* are found only as free-lying individuals, and the two species of *Terebratella* are either attached or free-lying. *Terebratella inconspicua* is predominantly attached to intertidal and subtidal rock outcrops whereas *T. sanguinea* is mainly free-lying. The relative densities of free-lying forms show that the most favored substrate is shell gravel in which each species occurs at average densities of 200 m^{-2}. On substrates of coarse sand *N. lenticularis* is the dominant species, whereas *T. sanguinea* dominates on muddy bottoms at an average density of 100 m^{-2} (Fig. 2); *T. inconspicua* and *N. lenticularis* each occur at densities of approximately 20 m^{-2} (*5*).

The distributions observed are thus not in accord with the belief that immobile suspension feeders are intolerant of muddy sediments or that free-lying and attached species are morphologically distinct. The question of the origin of the populations is also raised—whether free-lying populations are derived from those on nearby rock walls or recruited to dif-

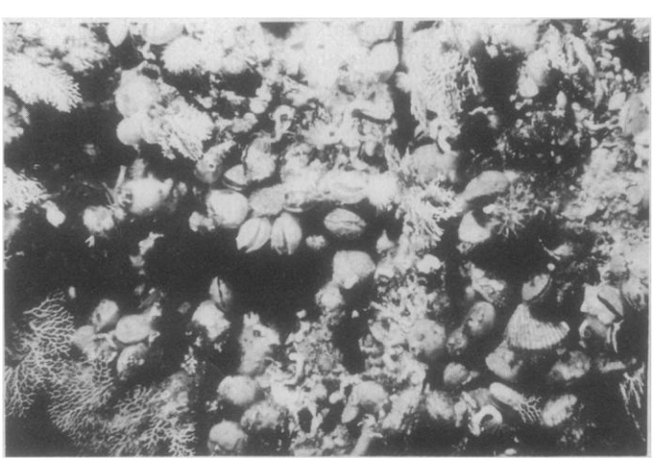

Fig. 1. In Long Sound, Preservation Inlet, a vertical rock wall (area ~ 1.0 m^2) at a depth of 20 m supports the brachiopods *Liothyrella neozelanica*, *Terebratella sanguinea*, and *Notosaria nigricans* and the stylasterine coral *Errina novaezelandiae*. [Photo by P. J. Hill]

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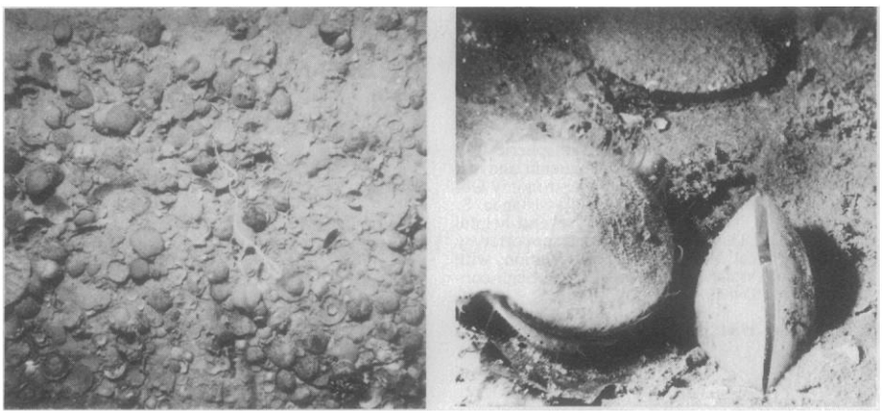

Fig. 2. The sea floor at Paterson Inlet, Stewart Island, at 30 m has a substrate of mud. (Left) The brachiopod *Terebratella sanguinea* and the bivalve *Chlamys gemmulata* are the dominant members of the epifauna. Area is ~ 1.0 m². [Photo by P. J. Hill] (Right) Close-up of *Chlamys gemmulata* and the brachiopod *Neothyris lenticularis*, the lophophore of which is visible through the gape. [Photo by R. J. Singleton]

ferent substrates. The origin of the populations and the authenticity of the observed distribution of species were determined by independent studies. Taxonomic studies of *N. nigricans* (*6*), of *N. lenticularis*, and of species of *Terebratella* (*7*) show that no specific differences occur in any of these species either between the geographical areas or between the free-lying and attached populations from the same area, Paterson Inlet. Identification of the material adhering to the tip of the pedicle of free-lying forms provided a guide to the substrates used as recruitment surfaces. For example, *T. sanguinea* occurs on rock substrates only as a solitary form, that is, individuals are not found attached to the shells of conspecies to form clusters. Therefore, the pedicles of free-lying individuals of *T. sanguinea* should retain fragments of rock, had they been dislodged from attachment sites on rock surfaces. Approximately 2000 free-lying individuals examined showed that fragments adhering to the pedicle were parts of the shells of those animals that predominate in the particular areas sampled. For example, the dominant living species in the muddy sediments of Paterson Inlet are *T. sanguinea* and the bivalve *Chlamys gemmulata* (Fig. 2, right); the whole and fragmented shells of these species are the common attachment sites for individuals of living populations. In addition, the study of living populations and death assemblages from soft substrates showed that the number of recruits and mortality rates account for the size of adult populations. These population studies show also that size-frequency histograms are distinctive for intertidal, subtidal, and benthic populations of *T. inconspicua* (Fig. 3) and that the same pattern is evident in benthic populations of *T. sanguinea* and *N. lenticularis*. The studies of populations that occur on soft substrates are of particular relevance to paleontology because the majority of fossil brachiopods lived in similar depositional environments and because the only populations of living brachiopods described are those of species that inhabit rocky shorelines (*8*).

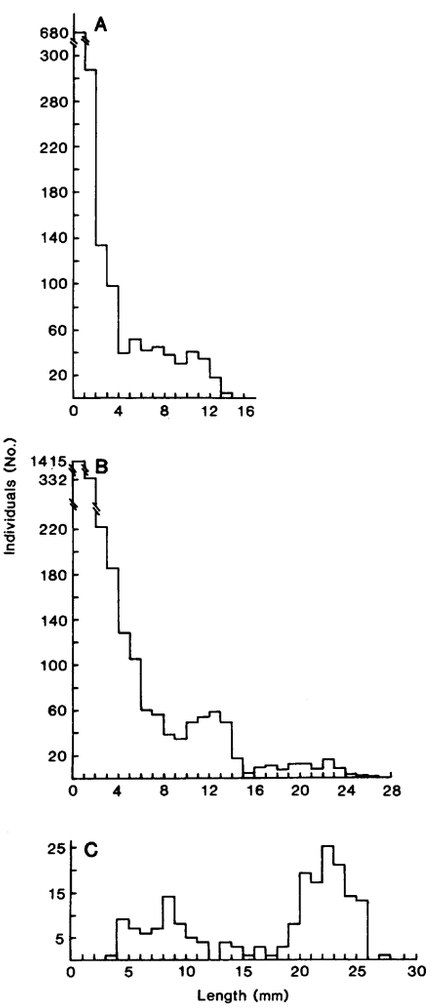

Fig. 3. Size-frequency distributions of *Terebratella inconspicua* samples from three habitats in Paterson Inlet, Stewart Island. Data from (*5*). (A) Intertidal area under stones (0.23 m²). (B) Subtidal areas with rock crevices at 7 m (0.16 m²). (C) Benthic area with muddy sand at 22 m (1.0 m²).

These studies thus confirm observations that some species occur in widely different habitats. Morphological studies (*9*) reveal that the range of habitats occupied by species is correlated with pedicle type, and the pedicle is uniform neither in structure nor in function. Species with "muscular" pedicles, *N. nigricans*, are restricted to large stable attachment surfaces while species with "transitional" pedicles, *Terebratella*, may be attached to or free-lying on a wide range of substrates. Thus no morphological barrier exists to the observed patterns of distribution. The assumption that living brachiopods do not occur on muddy sediments suggests a physiological barrier that was not evident in my survey. The contrasting environments studied, in which brachiopods are dominant members of the fauna, illustrate two factors. (i) For some species the adoption of an attached or free-lying existence is governed by the habitats available. (ii) The physical requirements of these brachiopods are such that they apparently function as efficiently attached to subtidal rock faces as they do when free-lying on mud (Fig. 2, right), an observation supported by the similarity of overall size and growth increments in populations studied from these two very different substrates (*7*).

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3. Free-lying is used as a synonym for free-living by some authors, and these terms are applied to fossil forms that bear no evidence of a pedicle foramen and are described as nonpediculate. Both terms are also used to describe the adult state of specimens of *Neothyris lenticularis* in which shell fragments (the larval attachment surface or its remnants) are found attached to the tip of the pedicle. F. Surlyk [*Biol. Medd. K. Dan. Vidensk. Selsk.* **19**, 48 (1972)] describes similar Cretaceous brachiopods, assumed to have been attached in young stages, as secondarily free-living forms. Hence the terms free-lying and free-living are applied both to nonpediculate fossil species and to living forms that have a pedicle but are effectively free-lying. Therefore, the basic difference between individuals designated attached or free-lying is the size of the attachment surface. A further distinction between these individuals is that the attachment surface and the substrate may not coincide. The

substrate in the attachment surface for individuals attached to rock faces, but free-lying individuals attached to shell fragments may occur on muddy substrates.

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Novel Complex Polar Lipids from the Methanogenic Archaebacterium *Methanospirillum hungatei*

Abstract. *The methanogenic archaebacterium* Methanospirillum hungatei *contains two unusual phosphoglycolipids that account for 64 percent of the total cellular lipids. These lipids are derivatives of the dibiphytanyl diglycerol tetraether, previously identified in methanogens. One of the free hydroxyls of this tetraether is esterified with glycerophosphoric acid, and the other is linked glycosidically to a disaccharide. The two phosphoglycolipids may function as covalently bonded lipid bilayers to impart stability and rigidity to methanogen membranes.*

Methanogenic bacteria are strict anaerobes, most of which utilize hydrogen and carbon dioxide to produce methane (*1*, *2*). Together with certain thermoacidophilic bacteria and the extremely halophilic bacteria, they are considered to represent a line of early divergence from the eukaryotic and eubacterial lines of evolutionary descent and are designated archaebacteria (*3*). Two recent reports (*4*, *5*) have shown that the lipids of methanogens are derived from phytanyl glycerol diether, previously identified in extremely halophilic bacteria (*6*), as well as from the biphytanyl diglycerol tetraether (C_{40}-tetraether), previously identified in thermoacidophiles (*7*). We now report the structural identification of the complex polar lipids in the methanogen *Methanospirillum hungatei* for comparison with those of extreme halophiles (*6*) and thermoacidophiles (*7*).

Seven polar lipid components were detected (Table 1): two phosphoglycolipids (PGL-I and PGL-II) derived from the C_{40}-tetraether; two glycolipids (DGT-I and DGT-II) derived from the C_{40}-tetraether; two glycolipids (DGD-I and DGD-II) derived from di-*O*-phytanyl glycerol; and a phospholipid (PG), the diphytanyl glycerol ether analog of phosphatidylglycerol. Both of the C_{40}-tetraether phosphoglycolipids are asymmetric structures with sugar residues on one side and the phosphoglycerol group on the other side of the tetraether moiety (Fig. 1).

Cells of *M. hungatei* were grown (*1*, *8*) and extracted by a modification (*9*) of the method of Bligh and Dyer (*10*). Total lipids (5.5 percent of cell, dry weight) were fractionated on a silicic acid column (Bio-sil A, 100–200 mesh) eluted with chloroform to remove neutral lipids, acetone to remove glycolipids, and a mixture of chloroform and methanol (3:2, by volume) to remove phosphoglycolipids and phospholipids. The individual polar lipid components were isolated in pure form by preparative thin-layer chromatography of the above fractions (see Table 1).

Lipids were hydrolyzed by first reacting them with anhydrous 2.5 percent methanolic HCl and then with 1*N* aqueous HCl; the water-soluble products (sugars and glycerophosphates) were identified by paper chromatography in a mixture of pyridine, ethyl acetate, and water (2:5:5, by volume, upper phase) (*9*). The lipid products (phytanyl diether and biphytanyl tetraether) were identified by thin-layer chromatography on silica gel G in a mixture of petroleum ether, ethyl ether, and acetic acid (50:50:1, by volume), by infrared and nuclear magnetic resonance (NMR) spectrometry, and by optical rotation and comparison with authentic standards (*6*, *7*). After hydrolysis of phosphoglycolipids in 1*N* methanolic NaOH, water-soluble products were analyzed for α-, β-, and *sn*-3 glycerophosphates (*11*, *12*), and lipid products were identified by thin-layer chromatography in a mixture of chloroform, methanol, acetic acid , and water (85:15:10:3, by volume). Phosphoglycolipids (free acid form) and glycolipids were completely methylated (*13*), and the partially methylated sugars were identified as their alditol acetates (*14*) by gas chromatography and mass spectrometry (*15*).

The pure, isolated components were characterized by retardation factor (R_f) values, staining behavior, presence of di- or tetraether, water-soluble hydrolysis products, and molar ratios of phosphate (*P*), sugars, and di- or tetraether (Table 1). The phosphoglycolipids PGL-I and PGL-II were derivatives of the tetraether ($[\alpha]_D$ + 8.7°; reported in (*16*), $[\alpha]_D$ + 7.5°) and contained *P*, hexose, and tetraether in the molar ratio 1:2:1. However, PGL-I contained 1 mole each of glucose and galactose, whereas PGL-II contained 2 moles of galactose. The glycolipids DGT-I and DGT-II also contained the tetraether and hexose (glucose and galactose in DGT-I and galactose only in DGT-II) in the molar ratio 1:2, and could be derived from PGL-I and PGL-II, respectively, by removal of the glycerophosphate moiety with 1*N* methanolic NaOH. The glycolipids DGD-I and DGD-II had the same hexose residues as DGT-I and DGT-II, respectively, but the lipid moiety was the *sn*-2,3-di-*O*-phytanyl diether instead of the tetraether (Table 1).

Complete methylation of PGL-I, DGT-I, or DGD-I yielded 2,3,4,6-tetramethylglucose and 3,5,6-trimethylgalactose, showing the presence of one terminal sugar, glucopyranose, which was linked to galactofuranose at the 2-position (compounds **1**, **2**, and **5** in Fig. 1). Methylation of PGL-II, DGT-II, or DGD-II, however, gave 2,3,5,6-tetramethylgalactose and 2,3,5-trimethylgalactose, showing that the terminal sugar was galactofuranose linked to another galactofuranose at the 6-position (compounds **3**, **4**, and **6** in Fig. 1). The ano-

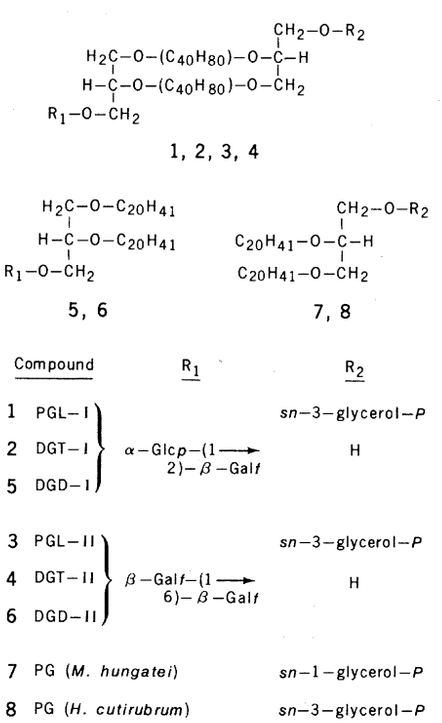

Fig. 1. Structures of complex lipids in *Methanospirillum hungatei*.

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