Cah. Biol. Mar. (1998) 39 : 259-262

Ectosymbiosis between filamentous sulphur bacteria and a stalked barnacle (Scalpellomorpha, Neolepadinae) from the Lau Back Arc Basin, Tonga

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Introduction

Biologists studying barnacles abundant at hydrothermal vents in the Pacific have been on the lookout for symbiosis with sulphur bacteria, since such a mode of life is predominant in other groups of vent animals, notably tube-worms and molluscs. However, neither ectosymbionts nor endosymbionts have been seen. The neolepadine barnacles from vents have more delicate cirri and mouth parts than scalpellomorphs from surrounding deep sea and shelf regions, features that can be regarded as adaptations to feeding on fine particles, including bacteria (Newman, 1979; Jones, 1993; Newman & Yamaguchi, 1995; Southward et al., 1997; Yamaguchi & Newman, 1997a, 1997b). But there has been no hint of strategically placed ectosymbiotic bacterial growths comparable to those on the bresiliid shrimp, *Rimicaris exoculata* Williams & Rona, 1986, from the Mid-Atlandic Ridge (MAR) (Pimenov et al., 1992; Segonzac, 1992; Segonzac et al., 1993; Gebruk et al., 1993).

We now record the occurrence, in an undescribed neolepadine from the Lau Back-Arc Basin, of cirral setae that are at least twice as long as those in other vent barnacles and bear growths of filamentous bacteria. The bacteria are comparable to those found on *Rimicaris* at the MAR.

Materials and methods

Two species of neolepadines, Lau A and Lau B, were collected by the IFREMER submersible 'Nautile' during the BIOLAU expedition, 1989, which discovered very diverse fauna at 22° S, 117° W (Desbruyères et al., 1994). The Lau sites have high temperature venting, rich in heavy metals (Fouquet et al., 1991). Specimens of Lau B were found peripheral to those of Lau A, and were sometimes associated with the sessile barnacle *Eochionelasmus ohtai* Yamaguchi, 1990 (Yamaguchi & Newman, 1997b). Lau A came from three dives; BL 03 at 1870 m, BL05 at 1887 m, and BL 07 at 1914 m. While adults and juveniles of Lau A occurred on basalt, they were also found attached to the tube-worms *Lamellibrachia columna* Southward, 1991 (Vestimentifera) and *Siphonobrachia lauensis* Southward, 1991 (Pogonophora, Perviata). Specimens on *Lamellibrachia* were photographed soon after preservation (Fig 1a) and showed browning of the peduncle and capitulum by ferrugineous material. An in situ underwater photograph (see Desbruyères et al., 1994, plate 1 d), shows several attached to basalt, with the cirri extended in what looks like diffuse flow from a fissure, with bacterial growths just visible on the large cirral setae.

Results

Externally, Lau A resembles other neolepadine barnacles from the East Pacific Rise, the South West Pacific (Lau, Fiji and Manus) and the South East Indian Ridge; the capitular armament ('shell plates') is quite typical for the group (Fig. 1b). However the Lau A species can develop a relatively long peduncle (stalk) and most individuals show the 'white' appearance of the cirri when these are protruded, the effect of the bacterial growths.

The barnacles were dissected to determine their taxonomic status, a matter which is still under consideration and requires detailed comparison with other undescribed neolepadines. It was immediately apparent that the setae of cirri 3 to 6 were coated with a white 'fuzz' (Fig. 1 c). Light microscopy showed the 'fuzz' to be growths of filamentous sulphur bacteria similar to those growing on the maxillipeds of *Rimicaris* from the MAR. In some specimens, which may recently have cast their exoskeletons, the bacterial filaments were sparse. In others, including one showing signs of

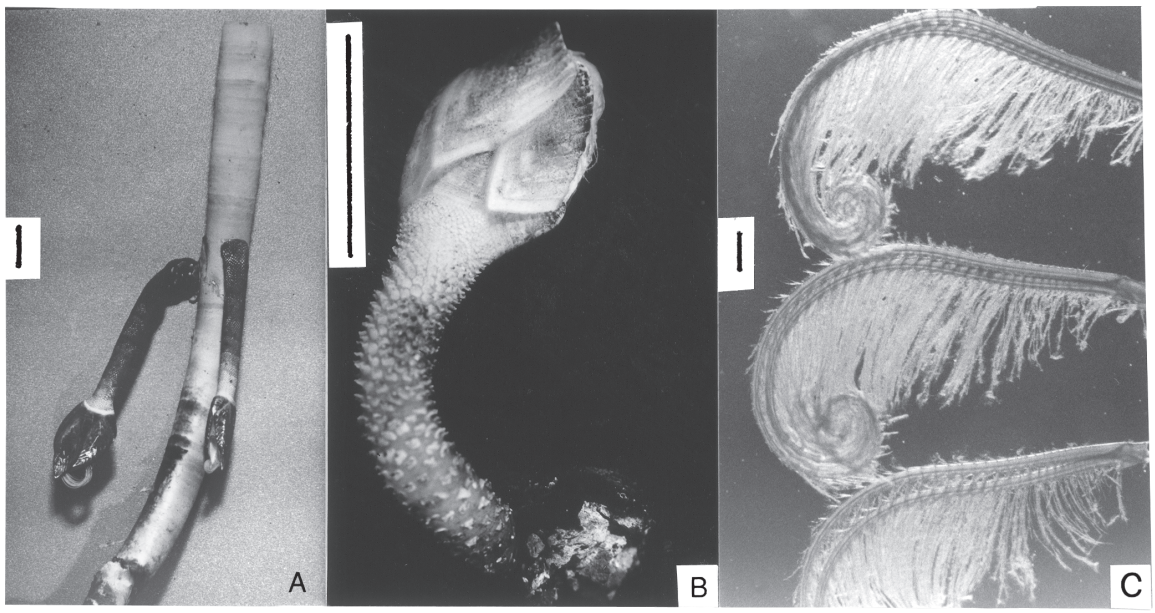

Figure 1. Lau barnacle species A. a) large, on upright tube of *Lamellibrachia columna,* hanging down; b) smaller, detached from basalt; c) cirri 4 to 6 on slide, seen by dark field illumination, showing long setae and their bacterial coating. Scale bars: a - 10 mm; b - 10 mm; c - 1 mm.

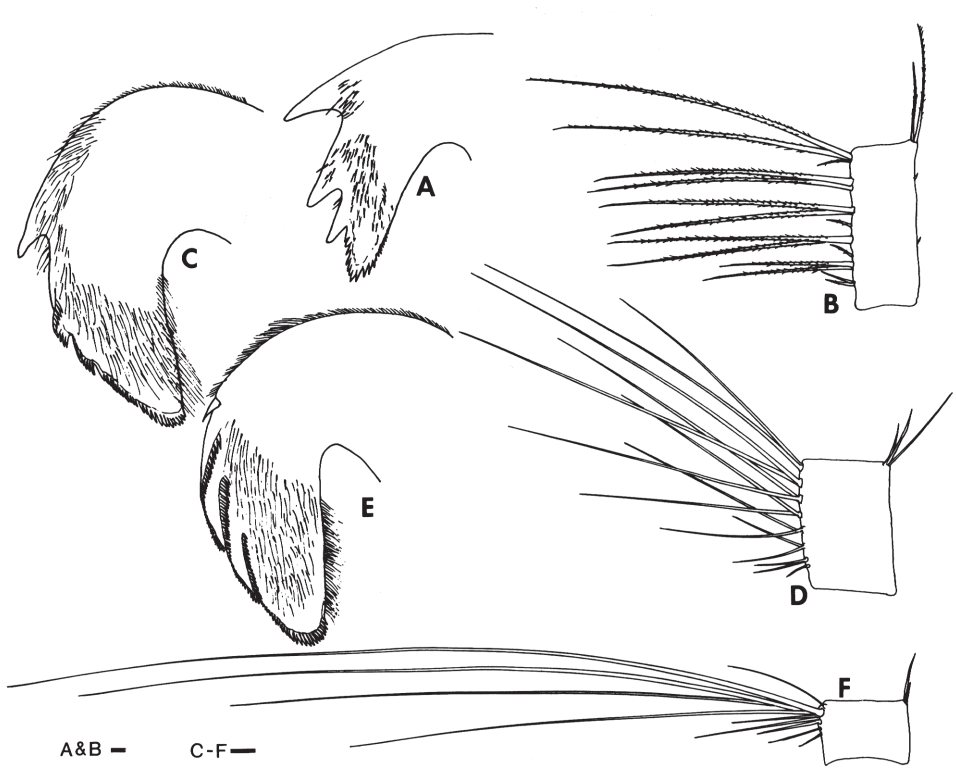

Figure 2. Mandibles and cirral setae of Lau A barnacle compared with other species. a) and b) from *Pilsbryscalpellum paralellogramma* (Hoek, 1883); c) and d) from *Neolepas* sp., Lihir Volcano, Papua New Guinea; e) and f) from Lau A. Scale bars 100 µm.

incipient ecdysis, the filaments were very dense and also occurred on the setae of cirri 1 and 2, making normal setose feeding by filtration of particles difficult. The smallest juvenile examined, 3 mm total length, had long setae but no bacterial growths.

The setae, especially those on cirri 3 to 6, are considerably elongated (Fig. 2 f). Comparison with other neolepadine barnacles equipped for normal setose feeding (Fig. 2 b, d) shows the setae are from 2 to 4 times as long in the Lau A species, with a setal/article ratio that is 1.5-2.3 times that of the other species (Table 1). The mouth parts of neolepadines, as well as other vent barnacles, are finely setose ('hairy'). The mandibles are basically tridentoid and the two lower teeth and the inferior angle have been converted into comb-like structures (Fig. 2 c, e) that contrast with the large puncturing spines of typical scalpellids (Fig. 2 a). The mandibles of Lau A differ from those in other vent barnacles in that these comb-like structures are three-quarters reflexed towards the interior surface; and the first or upper tooth is reduced to a simple spine held close to the body of the mandible (Fig. 2 e). This appears to be a further development for combing bacteria from the cirri.

Measurements of the bacteria were made on fixed material by light microscopy (Fig. 3 a, b) and by scanning electron microscopy (SEM) after critical point drying (Fig. 3 c). The bacterial filaments are mostly<100 µm long, but some are up to 220 µm in length. The "cells" constituting the filaments are about 1 µm long. A traverse of the SEM prints, taking in 30 filaments, gives a range from 0.25 to 1.5 µm diameter (mean 1.15 µm±1.27 SD). The filaments are attached in clusters to the setae, usually spreading out perpendicularly, but sometimes there are bacteria that form a thalloid coating of the setae from which the filaments arise. SEM did not show internal structure, but the boundaries of the 'cells' are sometimes visible as constrictions. From SEM, cirri 3 - 6 are without setules or other structures that might help hold the bacteria in place. Thus the main modification of Lau A for 'cultivation' of bacteria is the extended surfaces provided by the very long setae.

Table 1. Comparison of Lau A barnacle setae length with other neolepadine barnacles equipped for normal setae feeding.

Sample Location	Lengths in mm		Ratio
	article	seta	s/a
Lau sp. A	0.33	4.6	14.0
Lau sp. B	0.10	0.67	6.7
South-East Indian Ridge	0.18	1.7	9.4
Lihir volcano	0.30	1.85	6.2

Discussion

Although the Lau A neolepadine colonizes basalt in addition to worm tubes, its co-occurrence with a comparatively large vestimentiferan, *Lamellibrachia columna*, which has endosymbiotic bacteria, underlines the dependence of the community on sulphide. Compared to ordinary deep-sea scalpellomorphs that owe their carbon to photosynthetic sources, vent barnacles must derive their carbon partly from chemoautotrophic bacteria, to a degree depending on the closeness of the association with vent sources. In the case of Lau A we have a very close association between a vent barnacle and bacteria, suggestive of symbiosis, with the probability that the barnacle is dependent on the setal bacteria for much of its food.

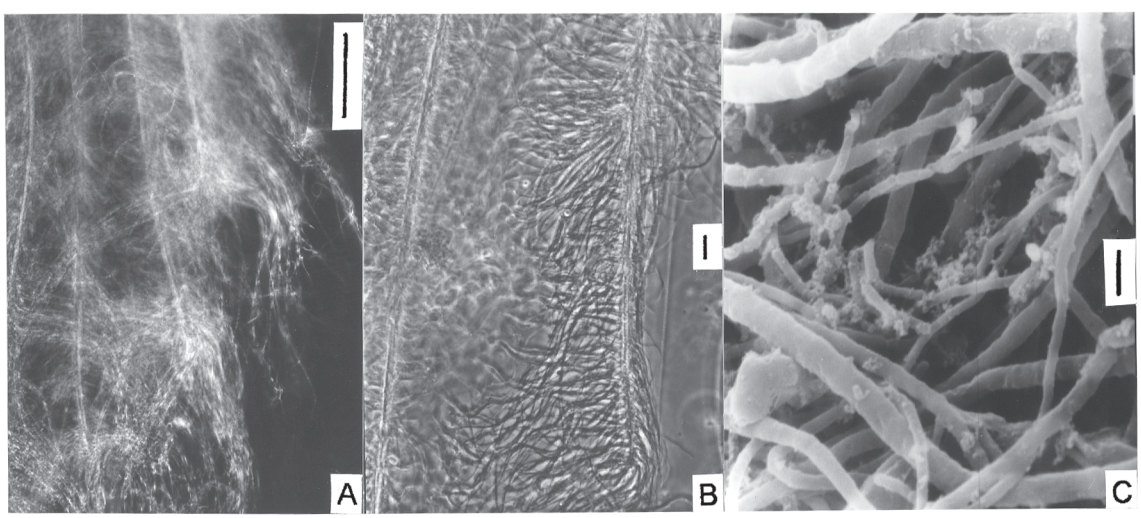

Figure 3. Bacteria on setae of Lau A. a) several setae, thickly coated, dark field illumination; b) bacteria on setae, phase contrast; c) scanning electron micrograph of bacteria. Scale bars: a - 100 µm; b - 10 µm; c - 1 µm.

New material is needed for further study of these animals. Expeditions finding stalked barnacles at vents or seeps are requested to handle them with great care, and preserve specimens by freezing and in 80% ethanol as soon as possible after collection.

Acknowledgements

We are grateful to IFREMER, Brest: Dr. A.-M. Alayse (chief scientist of the Biolau expedition), Dr. M. Segonzac and P. Briand of the sorting centre for giving us the opportunity to study these specimens, and for supply of photographs. Thanks are due to Dr. Eve Southward and Dr. Andrey Gebruk for advice and to Dr. Keith Ryan (Plymouth) and Charles Graham (SIO) for electron micrographs.

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