Gill-Cleaning Mechanisms of a Dendrobranchiate Shrimp, *Rimapenaeus similis* (Decapoda, Penaeidae): Description and Experimental Testing of Function

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ABSTRACT Observations on functional morphology and results from experiments demonstrate that setiferous epipods compose the major gill-cleaning mechanism in a penaeoid shrimp, *Rimapenaeus similis*. Epipods on the second maxillipeds and on pereopods 1–3 are equipped with long setae bearing an array of digitate scale setules. These multidenticulate setae reach to most gills and are jostled among them during limb movements. Experiments were performed in which epipods were removed from the gill chamber on one side (experimental) but not the other (control); treated animals were exposed to fouling in a recirculating water system for 2 weeks. Particulate fouling, measured by reduction in relative gill transparency, was significantly greater on experimental than control gills. The pereopodal exopods, not previously implicated in gill cleaning in any decapod, were similarly identified as important gill-cleaning structures. Equipped with long multidenticulate setae like those on the epipods, exopods sweep back and forth over the gill filaments just under the gill cover, areas not reached by the epipods. Exopod-ablation experiments were conducted that showed that exopods prevent particulate fouling on gill surfaces over which they sweep. The similarity in action of the passive gill-cleaning system of *R. similis* to that of crayfish (Bauer [1998] Invert Biol 117:29–143) suggests the hypothesis that the epipodal and exopodal cleaning setae of *R. similis* are ineffective against epibionts. The reduction in epipodal and exopodal cleaning systems that occurs in the Penaeoidea is hypothesized to be compensated for by increased development of gill-cleaning setae on the branchiostegite, scaphognathite, or other structures. J. Morphol. 242:125–139, 1999.

KEY WORDS: cleaning; epipods; exopods; fouling; gills; shrimp

One of the unique features of decapod crustaceans (e.g., shrimps, lobsters, crabs) is that the gills are enclosed in a branchial chamber. The advantages of gill enclosure are that the gills are protected from mechanical injury and, in the narrow confines of a branchial chamber, water can be pumped rapidly over gill filaments. However, there is a disadvantage to gill enclosure: the mass of gill filaments in a restricted space serves as a sediment trap (Bauer, '79, '89, '98). Particulate matter carried in by the inhalant water flow may be caught by or settle on gill filaments, covering their surfaces and preventing the gill functions of gas exchange, excretion, and ion regulation. The rapid flow of water past the gills also creates favorable conditions for the settlement and growth of microfouling organisms such as epibiotic bacteria and protistans. As a result, most decapods show adaptations for keeping gills free of fouling that accumulates between molts. In many caridean and stenopodidean shrimps, as well as anomuran crabs and callianassid "ghost shrimps," gills are cleaned by cheliped brushing. Specialized chelipeds with brushes of complex setae are inserted

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into the gill chamber and the decapod actively brushes and picks fouling material from the gills (Bauer, '79, '81, '89). The efficiency of cheliped brushing in keeping gills free of both epibiotic and particulate fouling has been demonstrated experimentally by Bauer ('79) in caridean shrimp and Pohle ('89) in an anomuran crab.

In other decapods, no such active gill-cleaning mechanism has been observed. However, there appear to be indirect or passive methods for cleaning gills in these groups. Setobranch setae arise from the coxal (most proximal) segment of the thoracic legs and are directed on and among the gill filaments. Upon movements of the thoracic limbs, these setae are jostled and thrust among the filaments, presumably cleaning them. Setobranch setae are found in many caridean shrimps, thalassinid and axiid thalassinideans, and in parastacoidean and astacoidean crayfishes. In other decapods, coxal outgrowths (epipods) positioned between gills bear setae similar in microstructure to setobranch setae and to those in cheliped gill brushes. These setiferous epipods, found in penaeoidean shrimps, palinurid and nephropid lobsters, and brachyuran crabs have been presumed to be passive gill-cleaning structures (Bauer, '89). Batang and Suzuki ('99) have recently described both setobranchs and setiferous epipods in the "mud lobster" *Thalassina anomala*. Other indirect gill-cleaning methods include multidenticulate setae fringing the scaphognathite (gill bailer) in some decapods (Bauer, '79; Suzuki and McLay, '98), as well as similar setae on the inner side of the branchiostegite (gill cover) in cambarid crayfishes (Bauer, '98). The only experimental study of the actual function of putative indirect gill-cleaning mechanisms was performed on the crayfish *Procambarus clarkii* (Bauer, '98). Setobranch and branchiostegal setae were shown to be very efficient at preventing sediment fouling, but they were not effective against microbial fouling.

The gill-cleaning mechanisms of a major decapod crustacean group, the Penaeoidea, have only been suggested (Young, '59; Bauer, '81, '89; Dall et al., '90) and have never been demonstrated experimentally. The objective of this study was to identify, to describe, and to test experimentally possible gill-cleaning mechanisms in a member of this group, the penaeid shrimp *Rimapenaeus similis*.

MATERIALS AND METHODS

Rimapenaeus similis (Smith, 1885) is a penaeid species formerly included in the genus *Trachypenaeus s.l.*, revised by Pérez Farfante and Kensley ('97). Specimens were collected by otter trawl in Mississippi Sound near Horn Island (30° 15′N, 88° 45′W) and the Pascagoula Ship Channel (30° 20′N, 88° 32′W), Mississippi, on several trips during 1991–1993. Live specimens used in observations and experiments were maintained individually on recirculating water tables with seawater at 25–30 ppt, water temperatures of 20–25°C, a 14/10hr day:night schedule, and daily feeding with commercial shrimp food in pellet form. Specimens used in morphological work were initially fixed in 10–15% seawater formalin, later washed with water, taken through a series of washes of 25%, 35%, and 50% ethanol up to final storage in 70% ethanol. Material used for scanning electron microscopy was taken through a graduated series from 70–100% ethanol, dried in CO_2 with an EMS 850 critical-point dryer, and sputter-coated with gold-palladium for 1–8 min at 10–20 nm/min, with longer coating times used for larger, topographically complex samples. Specimens were viewed with a JEOL 7000 FV scanning electron microscope at an accelerating voltage of 15 kv. Pérez Farfante and Kensley ('97), Pérez Farfante ('71), and Dall ('57) were followed in designation of gill type in *R. similis*.

Observations were made on living *Rimapenaeus similis* in order to observe grooming behaviors, especially possible brushing of gills by chelipeds or other appendages. Specimens were set up in recirculating aquaria with sand substrates. A total of 23 individuals were observed during the day for durations of 0.5–1.0 hr, for a total of 19 hr. Additional observations were taken from recordings made at night, when *R. similis* is most active, with a low-light, infrared-sensitive surveillance video camera, both at real time speed and a time-lapse speed of 10 pictures/sec (12-hr period on a single VHS videocassette), for a total of 90 hr on 17 individuals. Light for night recordings was supplied by infrared lamps (880 nm).

The possible gill-cleaning function of epipods and exopods was tested by removing them, in separate experiments, from the branchial chamber of one side of the shrimp but not the other. First attempts at ablation experiments were interrupted by molting of treated animals within a few days to a week of ablation. This problem was reduced by

performing the ablations 1–4 days after the molt of individually maintained shrimps, i.e., sufficient time for a shrimp to recover from a molt, but long enough so that several molted individuals could be ablated on a single day. Shrimps were anesthetized by temperature shock before ablations by changing them from water of ambient temperatures of 21–

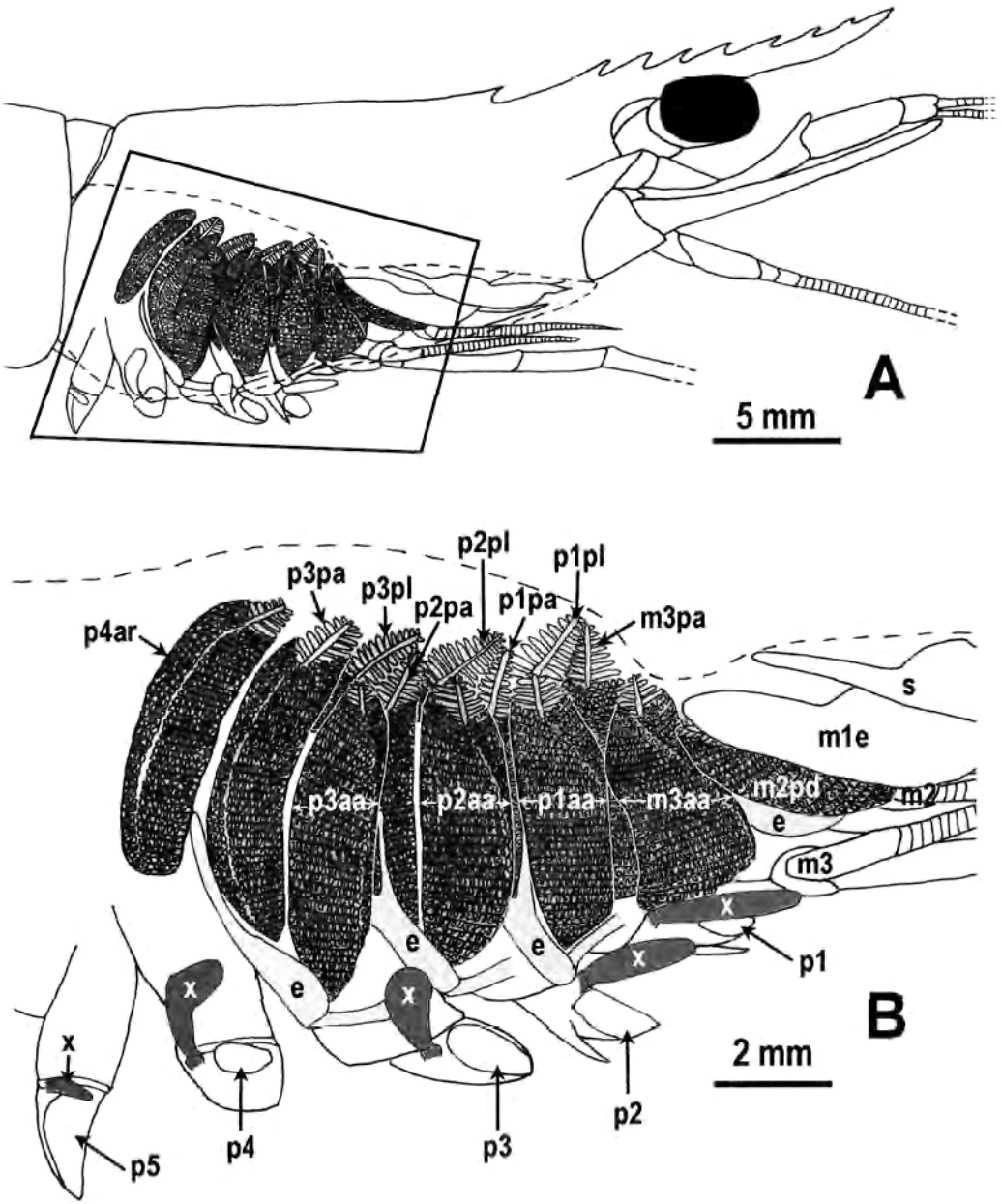

Fig. 1. *Rimapenaeus similis* **A:** Anterior end of shrimp, with branchiostegite or gill cover (dotted line) cut away to reveal gills. Area outlined by box is shown at higher magnification in **B** and Figure 2A,B. **B:** Area of gill chamber outlined by box in **A**, showing exterior view of undisturbed, complete set of gills, exopods, and epipods. aa, anterior arthrobranch; ar, single arthrobranch of pereopod 4; e, gill-cleaning epipod; m1e, epipod of first maxilliped; m2, maxilliped 2; m3, maxilliped 3; pa, posterior arthrobranch; pd, podobranch; p1–p5, pereopods 1–5 (arrows point to basi-ischial breakage plane, with distal portion of limb removed); s, scaphognathite (gill bailer); x, pereopodal exopod.

23°C to 12–15°C. Epipods or exopods were removed from the right side of anesthetized shrimps using fine forceps. Treated shrimps were then maintained individually on water tables and exposed to fouling for 14 days, after which they were anesthetized by chilling and then preserved and stored in 10–15% buffered seawater formalin.

Gill fouling was measured in 25 individuals randomly selected from those that had survived the 14-day duration of the experiment without molting (all 35 after epipod ablation; in the exopod ablation, 9 of 42 molted 1–2 days before the end of the experiment). Particulate fouling of a gill was quantified by measuring its relative transparency when mounted on a slide in a compound light microscope, as in Bauer ('98). A gill was mounted in water on a depression slide and covered with a cover slip. Standardized locations on a gill were viewed at 250x so that gill tissue completely filled the field of view. For the epipod-ablation experiment, all gills were examined from the third maxillipeds and third pereopods, located anteriorly and posteriorly, respectively, in the gill chamber. Measures of transparency were taken midway along the length of the gill. In the exopod-ablation experiments, readings were taken on the anterior arthrobranchs of the third maxillipeds and pereopods 1–3, halfway between the midpoint and proximal end of each gill.

Intensity of light passing through the gills was measured with a light meter, set at a range of 0–200 lux ± 4%, whose sensor was mounted on the phototube of the microscope. Relative (percent) transparency was defined as the reading of light intensity with the gill inside the field of view divided by the light intensity without the gill × 100. Settings of factors besides magnification (250x) that affected the intensity of light transmitted through the slide (rheostat, apertures of field and iris diaphragms, condenser) were standardized.

TABLE 1. Distribution of gills, epipods, and exopods in Rimapenaeus similis

Body Somite	7	8	9	10	11	12	13	14
Thoracic Somite	1	2	3	4	5	6	7	8
Limb	M1	M2	M3	P1	P2	P3	P4	P5
Pleurobranchs	0	0	1	1	1	1	0	0
Arthrobranchs	r	2	2	2	2	2	1	0
Podobranchs	0	1	0	0	0	0	0	0
Epipods	1b	1c	0	1c	1c	1c	0	0
Exopods	1f	1f	1f	1c	1c	1c	1c	v

b, broad blade, fringed with short, plumose setae; c, equipped with long setae studded with digitate scale setules, cleaning hypothesized as principal function; f, flagelliform, equipped with plumose setae; M, maxilliped; P, pereopod; r, rudimentary gill composed of small number of filaments; v = reduced in size, vestigial.

RESULTS

Gills and gill-cleaning structures

Removal of the branchiostegite (gill cover) exposes the gills within the branchial chamber (Figs. 1A,B, 3A, Table 1). The podobranch of maxilliped 2, the anterior arthrobranchs of maxilliped 3 and pereopods 1–3, the posterior arthrobranch of pereopod 3, and the single arthrobranch of pereopod 4 form a laterally-positioned *outer layer* of gills (Figs. 1B, 3A). Deeper within the branchial chamber, medial to the large anterior arthrobranchs of maxilliped 3 and pereopods 1–3, is an *inner layer* of gills, composed of the anterior and posterior arthrobranchs of maxilliped 2, the posterior arthrobranchs of maxilliped 3 and pereopods 1–2, and the pleurobranchs of maxilliped 3 and pereopods 1–3 (Figs. 2A, 3B).

Setiferous epipods have been hypothesized as gill-cleaning structures in penaeid shrimps (Young, '59 ; Bauer, '81, '89; Dall et al., '90). In *Rimapenaeus similis*, such epipods are found on maxilliped 2 and pereopods 1–3 (Figs. 1B, 2A,B, 3A–E, 4). Each arises from the coxal segment as a narrow horizontal strap, expanding into a large, thin blade that extends up among the gills (Figs. 3B–D, 4). The epipod blades are wide proximally but are slightly narrower distally, and bifurcate into narrow branches in pereopods 1 and 2 but continue without interruption in pereopod 3 and maxilliped 2 (Fig. 4). Epipods of pereopods 1–3 are inserted between the gills of the same segment and those of the next posterior segment (Figs. 1B, 2A, 3B–D,F). The epipod of maxilliped 2 is disposed somewhat more obliquely in the branchial chamber than the pereopodal epipods (Figs. 2A,B, 3E). Its broad proximal portion sits posterior to the three gills of maxilliped 2 and anterior and medial to the anterior arthrobranch of

Fig. 2. *Rimapenaeus similis*. Disposition of gill-cleaning epipods, exopods, and their setae. Area displayed is from box in Figure 1A, shown in Figure 1B with complete set of gills. **A:** Branchial chamber, with anterior arthrobranchs of maxilliped 3, pereopods 1–3 removed to reveal inner (medial) layer of gills and positions of gill-cleaning epipods. Anterior arthrobranch of maxilliped 2 is hidden medial to the posterior arthrobranch of that somite. **B:** Branchial chamber, all gills removed, showing disposition of multidenticulate setae of gill-cleaning epipods and exopods. aa, anterior arthrobranch; ar, single arthrobranch of pereopod 4; e, gill-cleaning epipod; m1e, epipod of first maxilliped; m2, maxilliped 2; m3, maxilliped 3; pa, posterior arthrobranch; pd, podobranch; p1–p5, pereopods 1–5 (arrows point to basi-ischial breakage plane, with distal portion of limb removed); s, scaphognathite (gill bailer); x, pereopodal exopod. Unmarked arrows point to the attachment stalk of the anterior arthrobranchs which were removed.

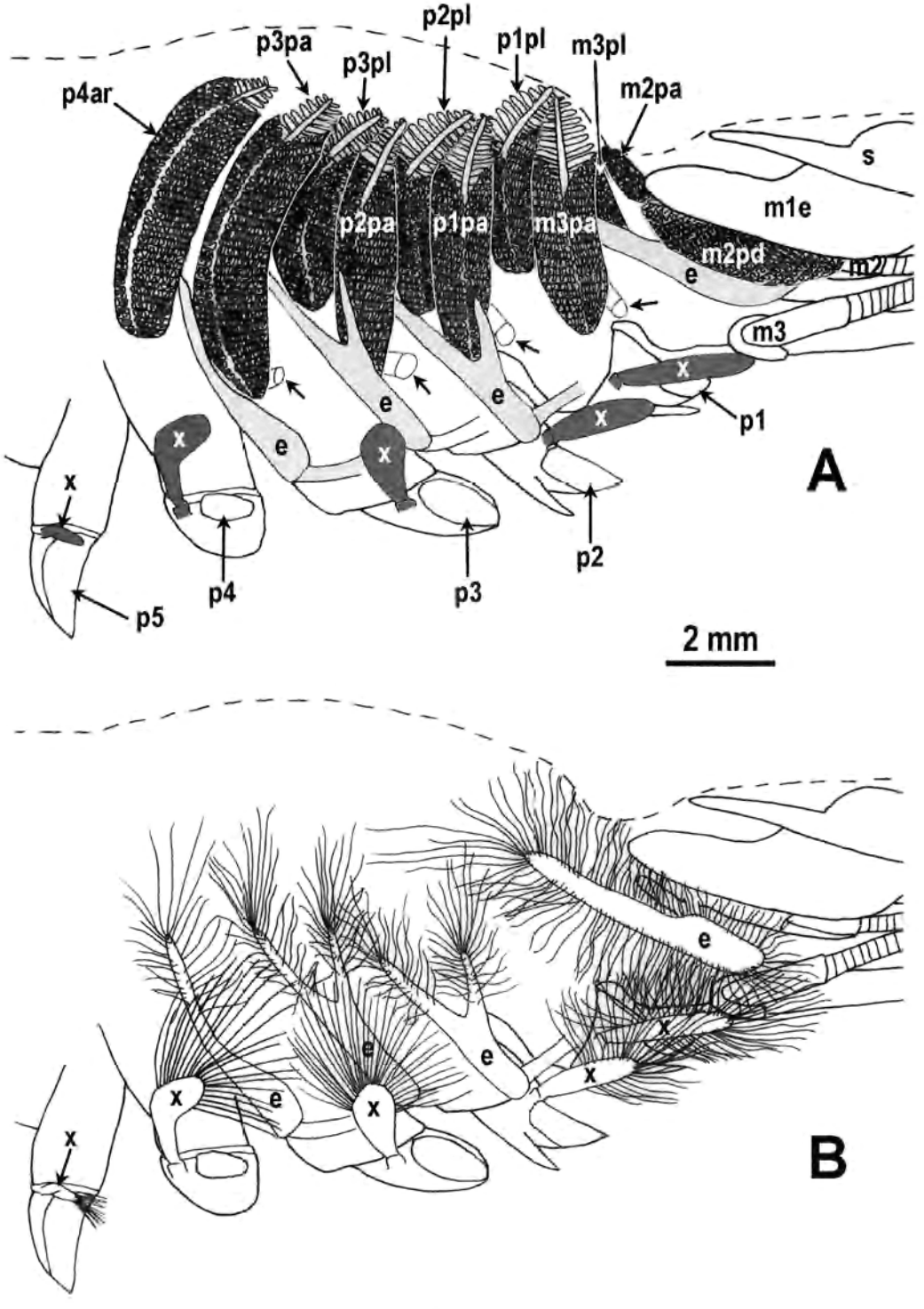
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Figure 2.

maxilliped 3. Its long distal portion continues medial to both arthrobranchs and lateral to the pleurobranch of maxilliped 3, terminating between the latter and the pleurobranch of pereopod 1 (Figs. 2A,B, 3E). The epipod of maxilliped 1 is a broad blade (Fig. 4) situated horizontally ventral to the scaphognathite (gill bailer) (Figs. 1B, 2A); its outer edge lies against the anterior side of the podobranch of maxilliped 2 (Figs. 1B, 2A, 3A).

The epipods of peropods 1–3 and maxilliped 2 are equipped with long, structurally complex setae (Figs. 2B, 3B,D–F, 5A–C,E,F). These setae are located distally on the part of the epipods of pereopods 1–3 that makes contact with the gills (Figs. 2B, 3B–D). The epipod of maxilliped 2, completely surrounded by gills, has these setae throughout its length (Figs. 2B, 3B,E). Arising from deep sockets (Fig. 5D), these multidenticulate setae are naked proximally and studded distally by an array of digitate scale setules (Fig. 5E,F). The setae extend among the gills and gill filaments (Figs. 3F, 5A–C). Due to the location of the epipods, nearly all gills are in contact with multidenticulate epipod setae. Setae of a pereopodal epipod make contact with the anterior sides of arthrobranchs of the immediately posterior somite and with the posterior sides of the arthrobranchs of the same somite. They extend medial to these same gills, as well as lateral to the pleurobranchs of the posterior somite. The coverage given by the longer maxilliped 2 epipod is even more extensive. Its setae make contact with all the gills of its own somite, including the outer or lateral side of its podobranch, with the anterior side and medial sides of both arthrobranchs of maxilliped 3, and with the pleurobranchs of maxlliped 3 and pereopod 1. The epipod of maxilliped 1 lacks the array of long complex setae found on other thoracic limbs; its outer edge is fringed by short plumose setae (Fig. 3E). However, on the underside of its outer edge, which lies against the anterior side of the podobranch of maxilliped 2, there is a scattering of relatively short setae equipped with denticulate scale setules.

Any movement of an appendage that carries a setiferous epipod causes the epipod and its setae to be moved among and over the gill filaments. Multidenticulate setae thus are scraped over and jostled among the gill filaments. Observations on living shrimps showed that the epipods of pereopods 1–3 do not move spontaneously, only with movement of the limb to which they are attached. The epipod of maxilliped 2 was observed to beat or rock within the gill chamber when the second maxilliped was not in motion. Microscopic observation of an acid-fuchsin-stained mount of this thin-walled structure indicates that this epipod, like those on the pereopods, lacks muscle fibers. Its movement may have been due to its proximity to the scaphognathite, whose constant movement draws water into the branchial chamber.

Epipodal setae with denticulate scales come into contact with almost all areas of the gills except for the lateral (outer) sides of the outer layer of gills, specifically, the arthrobranch of pereopod 4, the posterior arthrobranch of pereopod 3, and the anterior arthrobranchs of pereopods 1–3 and maxilliped 3. In looking for possible gill-cleaning setae associated with these gill areas, it was observed that the exopods of pereopods 1–4 (Figs. 1B, 2A,B, 3A,C, 6A,B) are bordered by long multidenticulate setae, arising from deep sockets, with similar microstructure (Fig. 6B) as that of the setiferous epipods. These pereopodal exopods are short, oblong, flattened structures (Fig. 7) that have been observed to rock back and forth, with their long multidenticulate setae sweeping over the lateral surfaces of those gills in the outer layer (except for the arthrobranch of pereopod 4 and the podobranch of maxilliped 2). The setae of these exopods extend up about as far as the proximal half to two-thirds of the gill (Figs. 2B, 3A,C, 6A). The exopod of a given limb sweeps the outer gill(s) of its somite when moved posteriorly (extended) and that of the anterior somite when moved anteriorly (flexed). There is a very small (vestigial) exopod on pereopod 5 (Figs. 1B, 2A,B, 7) equipped only with short plumose setae (Figs. 2B, 6C). The exopods of maxillipeds 1–3 are long and flagelliform (Figs. 1A,B, 6D, 7), especially those of maxillipeds 2 and 3, and all carry long plumose setae (Fig. 6D). Neither the exopods of pereopod 5 and maxillipeds 1–3 nor their setae come into contact with gills (Figs. 1A, 2A,B).

The scaphognathite and the inner surface of the branchiostegite, structures observed to have gill-cleaning setae in some other decapods, were examined. No long multidenticulate setae were observed extending over the gills from the posterior edge of the scaphognathite; instead, only short plumose setae occur there (Fig. 3E). The inner surface of the branchiostegite is largely devoid of setae with the exception of a group located posteriorly (Fig. 8A). Setae from this group arise from an area of the branchiostegite that lies directly against the arthrobranch of pereopod 4, a gill which has no contact with either epipodal or exopodal setae. In the dorsal part of the setal patch, the setae (Fig.

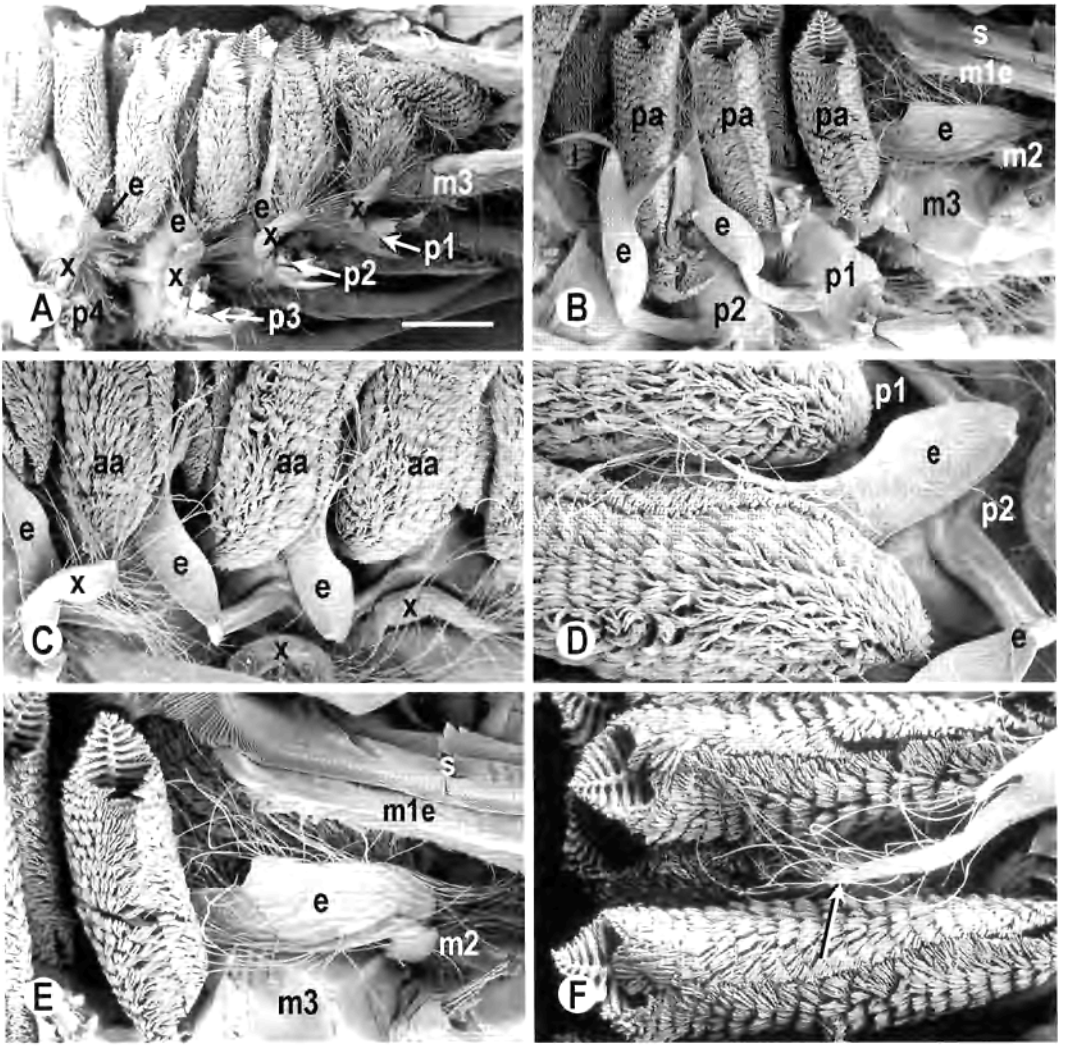

Fig. 3. *Rimapenaeus similis*. Branchial chamber and epipods. **A:** Branchial chamber with gill cover (branchiostegite) removed to reveal gills, basal portion of epipods, and exopods (cf. Fig. 1A,B); dorsal at top. **B:** Anterior end of branchial chamber, anterior arthrobranchs and maxilliped 2 podobranch removed to reveal posterior arthrobranchs, other underlying gills, and epipods of maxilliped 2 and pereopods 1 and 2 (cf. Fig. 2A), exopods also removed for clarity; dorsal at top. **C:** Arrangement of exopods, epipods, and gills on pereopods 1–3 upon removal of gill cover; dorsal at top. **D:** Blade of epipod of pereopod 1 extending under and between gills of pereopods 1 and 2, stem of epipod of pereopod 2 arising from the latter's coxal segment; dorsal to left. **E:** Anterior end of gill chamber (upper right in B), with anterior arthrobranch of maxilliped 3 and podobranch of maxilliped 2 removed to reveal proximal portion of setiferous epipod of maxilliped 2, with distal part extending under (medial to) posterior arthrobranch of maxilliped 3; dorsal at top. **F:** Setiferous distal branch (arrow) of epipod of pereopod 1 extending between posterior arthrobranchs of pereopods 1 and 2 (anterior arthrobranchs removed, dorsal to left). aa, anterior arthrobranch; e, gill-cleaning epipod; m1e, epipod of first maxilliped; m2, maxilliped 2; m3, maxilliped 3; pa, posterior arthrobranch; p1–p3, pereopods 1–3; s, scaphognathite (gill bailer); x, pereopodal exopod. Scale bar = 1.8 mm in **A**, 1.0 mm in **B**, 800 µm in **C**, 440 µm in **D**, 600 µm in **E**, and 540 µm in **F**.

8B,C), arising from deep sockets, are densely covered with long slender setules proximally (Fig. 8B,C) but distally with digitate scale setules (Fig. 8C,D). From dorsal to ventral in this setal group, the setae change in type from plumodenticulate to multidenticulate, with digitate scale setules only, similar to those described above on pereopodal exopods and epipods. There is a sparse scattering of very short but typical multidenticulate setae more anteriorly on the inner side of the branchiostegite (Fig. 8A).

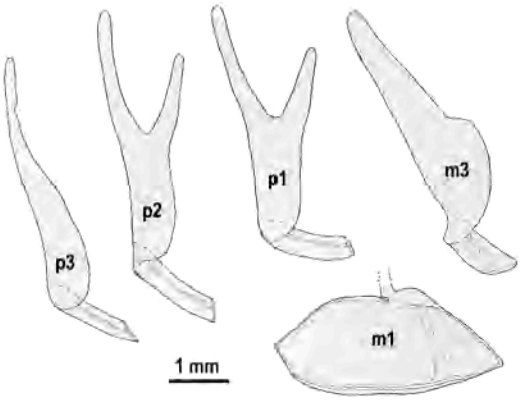

Fig. 4. *Rimapenaeus similis*. Relative size and shape of thoracic epipods. m1,3, epipods of maxillipeds 1,3; p1–p3, epipods of pereopods 1–3.

Observation of living shrimps for possible gill-brushing behavior

The activities of 40 *Rimapenaeus similis* individuals were observed for a total of 19 hr during the daytime and 90 hr at night, when this species is most active. Antennular and antennal grooming behaviors with the third maxillipeds and first pereopods were observed, as well as infrequent bouts of picking at various parts of the body with the chelipeds (pereopods 1–3). There was never any insertion of the chelipeds into the gill chamber nor any other sign of gill brushing by these appendages.

Experimental testing of gill-cleaning function

In the epipod-ablation experiment, epipods were removed from the branchial chamber of one side (experimental) but not the other (control) before treated shrimps were exposed to fouling on a recirculating water table. Treated shrimps preserved after the experiment were first examined by removal of the gill cover and observation through the dissecting microscope. There was no visible sign of fouling on the lateral surfaces of the outer gills (anterior arthrobranchs) when the branchiostegite was removed in either the experimental or control chambers. However, when the outer gills were removed from the experimental gill chamber, particulate fouling could be readily observed on the inner gills that lie medial to them, as well as on their own anterior and posterior edges, and especially on their medial sides. Qualitatively, particulate fouling on gills from the control gill chamber was difficult to observe. When gills were mounted for quantitative measurements (see below), less adherent particles could be observed being dislodged from experimental gills but not control gills, which were little fouled. Examination of equivalent control and experimental gills by scanning electron microscopy revealed the heavier fouling on experimental gills compared to little or none on controls (Fig. 9A–F), with fouling particles trapped among branches of gill filaments (Fig. 9C,E). Bacterial or other epibiotic fouling was not noticeable on either experimental or control gills, except perhaps for that associated with fouling aggregates on experimental gills.

Particulate fouling was quantitatively compared between control and experimental gills by measuring their degree of transparency to light, which is lowered by fouling. Medians of percent transparency for experimental gills were always lower than those of control gills, and there was only slight overlap in 95% confidence limits on medians for the anterior arthrobranchs (Fig. 10). The null hypothesis of no difference between experimentals and controls was tested for each pair of gills with the Wilcoxon matched-pairs signed-ranks test and was rejected in all cases ($P \leq 0.002$).

In the exopod-ablation experiment, particulate fouling could be readily observed on the lateral (outer) surfaces of the anterior arthrobranchs of the outer gills (maxilliped 3 and pereopods 1–3) in the experimental but not in the control gill chambers. When gills were mounted in order to measure light transparency, it was observed that there was usually a gradient of fouling in the experimentals, with more fouling proximally. However, the distal end of the gill also had noticeable fouling in the experimental gills but not in controls (Fig. 11). As in the epipod-ablation experiment, fouling was particulate, with little observation of epibiotic fouling on control or experimental gill filaments. Medians of relative gill transparency were lower in experimental gills than in controls, and there was overlap in 95% confidence limits only for the anterior arthrobranchs of the third maxillipeds (Fig. 12). However, the null hypothesis of no difference in relative transparency between control and experimental pairs of gills was rejected in all cases (Wilcoxon test, $P \leq 0.004$ in all comparisons).

DISCUSSION

The gill-cleaning system of *Rimapenaeus similis* is composed of a combination of structures that carry multidenticulate setae. The primary mechanism consists of the setiferous epipods of maxilliped 2 and pereopods 1–3. Their long multidenticulate setae are

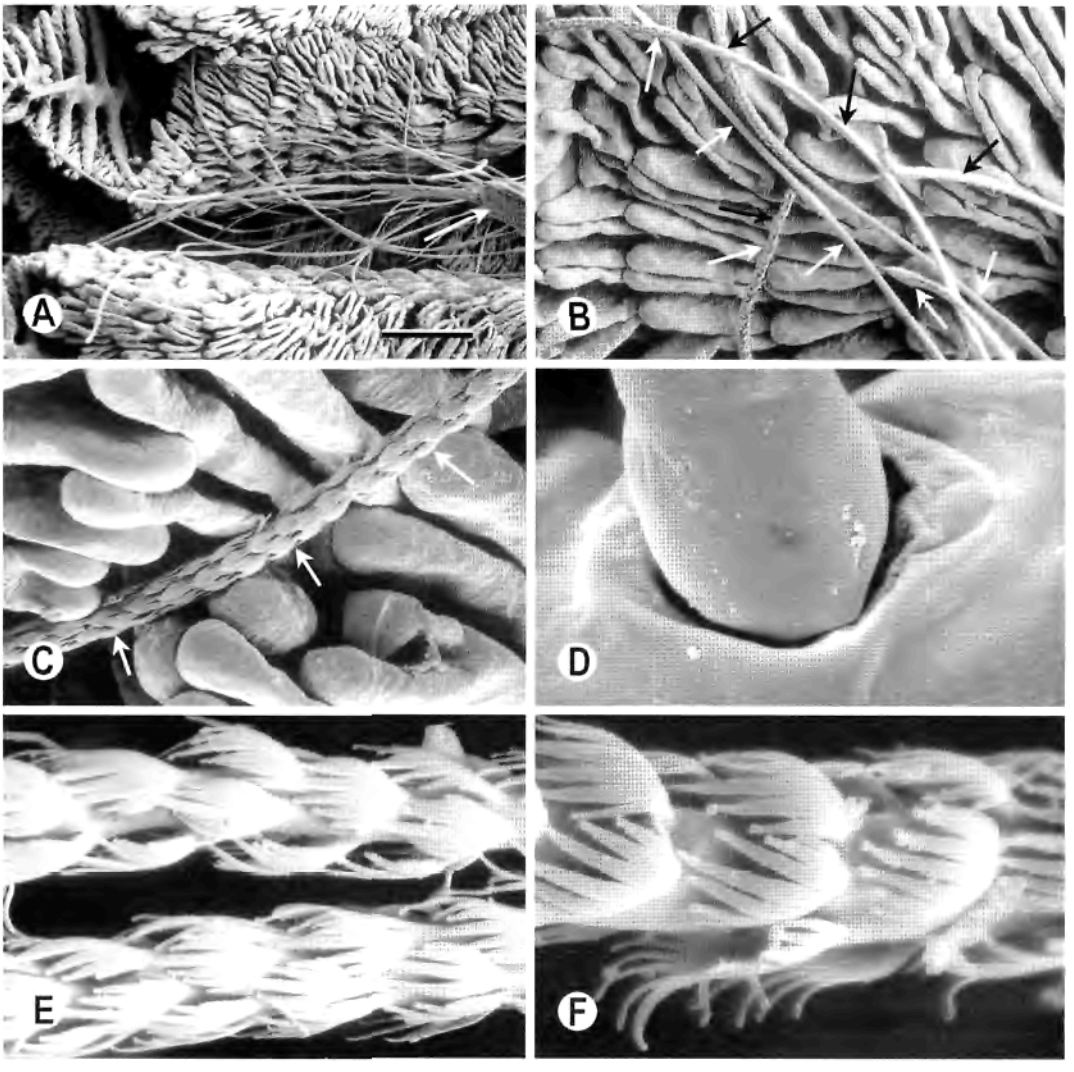

Fig. 5. *Rimapenaeus similis*. Multidenticulate epipodal setae. **A:** Setae at distal end (arrow) of pereopodal epipod in contact with adjacent posterior arthrobranchs (top, bottom) as well as to a pleurobranch (between and medial to the arthrobranchs) (dorsal to left). **B:** Epipodal setae (arrows) in contact with gill filaments. **C:** Single epipodal seta (arrows) among gill filaments. **D:** Base and deep socket of epipodal seta. **E:** Portions of two epipodal setae; note digitate scale setules on setal shafts. **F:** Digitate scale setules on shaft of epipodal seta. Scale bar = 230 µm in **A**, 80 µm in **B**, 20 µm in **C**, 5 µm in **D**, 4 µm in **E**, and 3 µm in **F**.

covered with digitate scale setules very similar in microstructure to setae that have been shown experimentally to clean the gills in a variety of decapods, e.g., cheliped brushes of caridean shrimps (Bauer, '79) and setobranch setae in crayfish (Bauer, '98). In *R. similis*, when the epipods are moved passively or indirectly by feeding or locomotory movements of the limbs that bear them, their setae are jostled among and over the gill filaments with which they make contact, preventing particulate matter carried in by the inhalant water from settling or remaining on respiratory surfaces. This gill-cleaning function of epipods is confirmed by the fouling of gills that occurs when epipods are removed. In the crayfish *Procambarus clarkii*, Bauer ('98) showed that setobranch setae, arising directly from the limb coxa, cleaned a comparable inner layer of gills.

Although epipodal setae clean most gill areas in *Rimapenaeus similis*, they do not reach to the lateral surfaces of the outer layer of gills, i.e., the anterior arthrobranchs

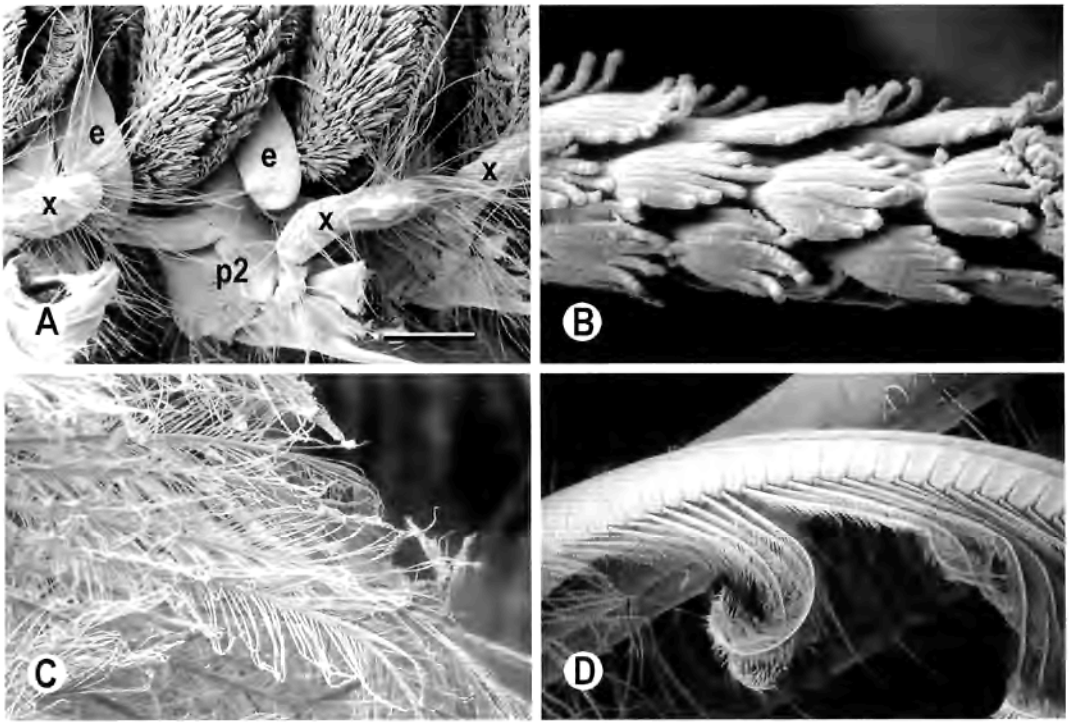

Fig. 6. *Rimapenaeus similis*. Exopods and setae. **A:** Pereopodal exopods equipped with multidenticulate setae extending over basal part of outer gills (anterior arthrobranchs). **B:** Portion of multidenticulate exopodal seta with digitate scale setules. **C:** Plumose setae on vestigial exopod of pereopod 5. **D:** Flagelliform exopod of third maxilliped with plumose setae. e, epipod; p2, coxa of pereopod 2; x, pereopodal exopod. Scale bar = 800 μm in **A**, 4 μm in B, 70 μm in **C**, and 220 μm in **D**.

of maxilliped 3 and pereopods 1–3, the posterior arthrobranch of pereopod 3, and the single arthrobranch of pereopod 4. Except for pereopod 4 arthrobranch, the lateral surfaces of these gills are swept by multidenticulate setae on specialized exopods of pereopods 1–4. Fouling of these areas did not occur when epipods (but not exopods) were experimentally removed, but sediment and particles did accumulate when the exopods (but not epipods) were removed. The primitive, flagelliform-type of exopod equipped with plumose setae, with a natatory or current-producing function, is retained on maxillipeds 2–3. In pereopods 1–4, however, the exopod has been modified into a shorter, flattened form, equipped with long, multidenticulate cleaning setae that sweep back and forth over the gills. The motion of these exopods is a retention of ancestral natatory movements used instead for gill cleaning. Interestingly, the very small exopod of pereopod 5, vestigial as far as size and any obvious function are concerned, retains the plumose setae of a primitive, natatory exopod.

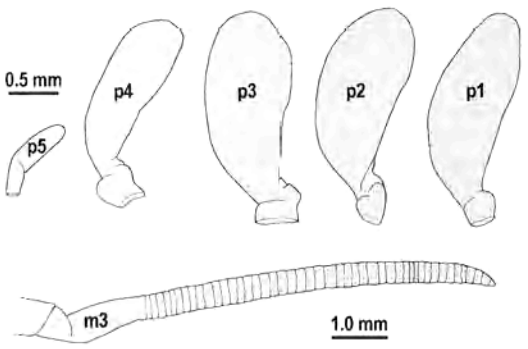

Fig. 7. *Rimapenaeus similis*. Relative size and shape of exopods. m3, flagelliform exopod of third maxilliped; p1–p5, exopods of pereopods 1–5.

In the crayfish *Procambarus clarkii*, which lacks pereopodal exopods, Bauer ('98) showed that gill surfaces directly medial to the branchiostegite (gill cover) were cleaned by a dense field of multidenticulate setae arising from the inner side of the branchiostegite itself. In *Rimapenaeus similis*, the branchiostegite is largely devoid of such cleaning setae (cf. Fig. 8A with Fig. 5B in Bauer, '98), and it is the

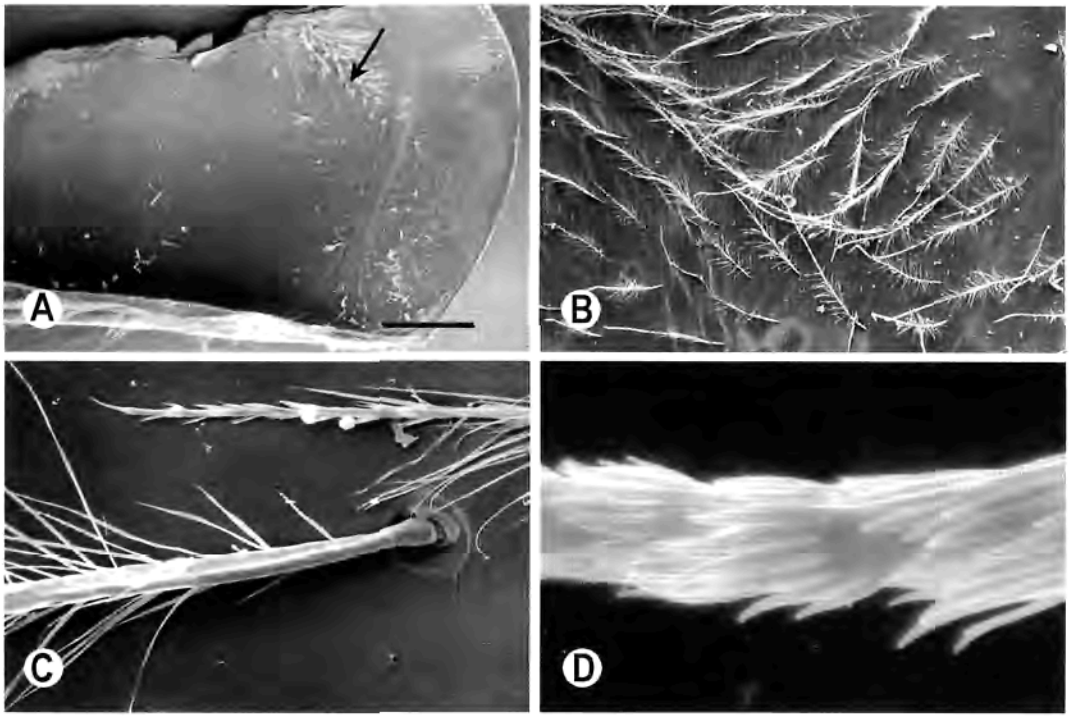

Fig. 8. *Rimapenaeus similis*. Cleaning setae of the branchiostegite (gill cover). **A:** Inner side of right branchiostegite, showing patch of setae (arrow, upper right) in contact with the arthrobranch of pereopod 4. **B:** Higher magnification of plumodenticulate setae indicated in **A**. **C:** Distal (above) and proximal (below) portions of setae located in patch indicated by arrow in **A**. **D:** Multidenticulate scale setules on distal ends of setae from the inner side of the branchiostegite. Scale bar = 1.4 mm in **A**, 300 μm in **B**, 20 μm in **C**, and 2 μm in **D**.

exopods of pereopods 1–4 that clean the gill surfaces just medial to the branchiostegite.

A few specialized setal groups appear to clean some limited, very specific gill areas. The only gill that is not in contact with exopodal and epipodal setae, the single arthrobranch of pereopod 4, appears to be cleaned by the sole field of setae on the inside of the branchiostegite. These setae, located directly opposite the pereopod 4 arthrobranch, bear digitate scale setules distally, protrude into the gill, and presumably jostle among its gill filaments during body movements.

Although the other thoracic epipods are gill-cleaning structures, the epipod of the first maxilliped primarily serves not in cleaning but rather as part of an anterodorsal funnel through which respiratory water enters the gill chamber in penaeids (Young, '59; Dall et al., '90). It is not equipped with long setae as are the other thoracic epipods; however, where it lies against the anterior side of the podobranch of its somite, there is a minor field of typical multidenticulate cleaning setae. Presumably, movements of the epipod, either inherent or caused by the beating of the scaphognathite above it, would cause these setae to clean the gill filaments below.

The scaphognathite sweeps long multidenticulate setae over the lateral surface of gills in some decapods, e.g., a few caridean shrimps (Bauer, '79; Suzuki and McLay, '98) and a thalassinid "mud lobster" (Batang and Suzuki, '99), and these scaphognathite setae are a probable passive cleaning mechanism. No such setae were observed on the scaphognathite in *Rimapenaeus similis*, in which the long exopodal cleaning setae sweep the lateral surface of the outer layer of the gills. In the crayfish *Procambarus clarkii*, which also lack scaphognathite setae, another passive mechanism, multidenticulate cleaning setae on the inside of the gill cover, cleans these gill surfaces, as discussed above. In *R. similis*, these are only developed over the most posterior gill, which is not reached by either exopodal nor epipodal cleaning setae.

Active brushing of the gills by chelipeds is the gill-cleaning mechanism of some caridean groups, anomuran crabs, and callianassids. Chelipeds are inserted among the gills and brushed vigorously among them. The

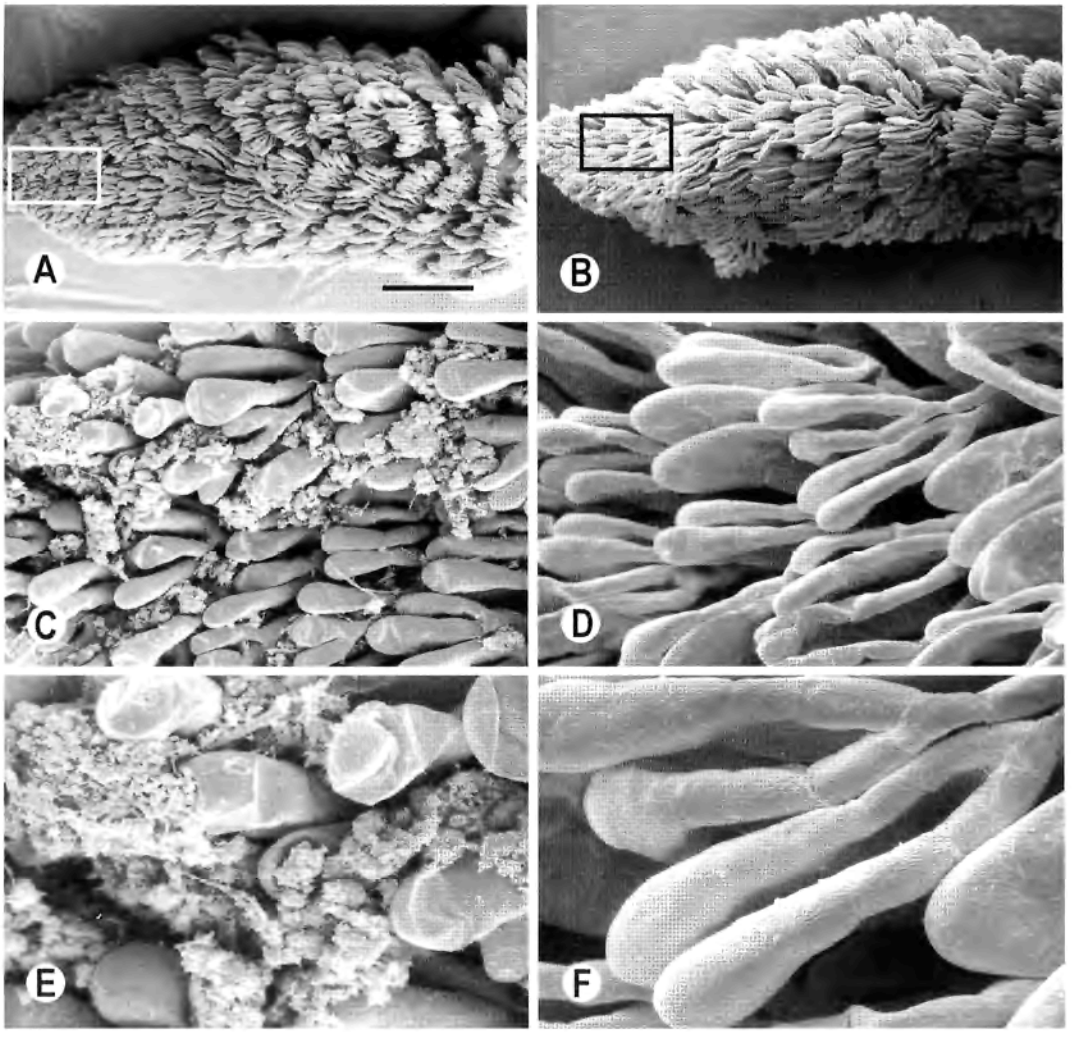

Fig. 9. *Rimapenaeus similis*. Results of the epipod-ablation experiment: comparison of fouling from experimental and control gills of the same specimen. **A:** Medial side of anterior arthrobranch (distal to left) of third maxilliped from experimental branchial chamber. Box *shows region of gill magnified in* **C** *and* **E**. **B:** *Same* gill type and view as **A**, but from control branchial chamber. Box shows region of gill magnified in **D** and **F**. **C:** Particulate fouling among filaments of experimental gill (compare with **D**) shown in **A. D**: Lack of particulate fouling among filaments from control gill (compare with **C**) shown in **B. E:** Higher magnification of particulate fouling on experimental gill (compare with **F**) from **A** and **C**. **F:** Higher magnification of clean filaments from *control gill (compare with* **E***) shown in* **B** *and* **D**. *Scale* bar = 360 µm in **A** and **B**, 60 µm in **C** and **D**, and 25 µm in **E** and **F**.

animal can selectively pick off material using fingers of the chelae, and it can concentrate its efforts on one area over another. Not surprisingly, cheliped cleaning of gills has been shown experimentally to be quite efficient at preventing both particulate and epibiotic fouling on the gills (Bauer, '79; Pohle, '89). Passive gill-cleaning mechanisms are certainly effective in preventing the accumulation of sediment and detrital particles from accumulating on gill surfaces, where such fouling may interfere with gas exchange, ion exchange, and excretion. In this study, significant particulate fouling occurred on gills when the passive cleaning mechanisms (exopods, epipods) were removed. Similarly, in the crayfish *Procambarus clarkii*, massive particulate fouling occurred in the experimental gill chamber when the setobranchs, the primary gill-cleaning structures, were removed from crayfish exposed to fouling in commercial ponds

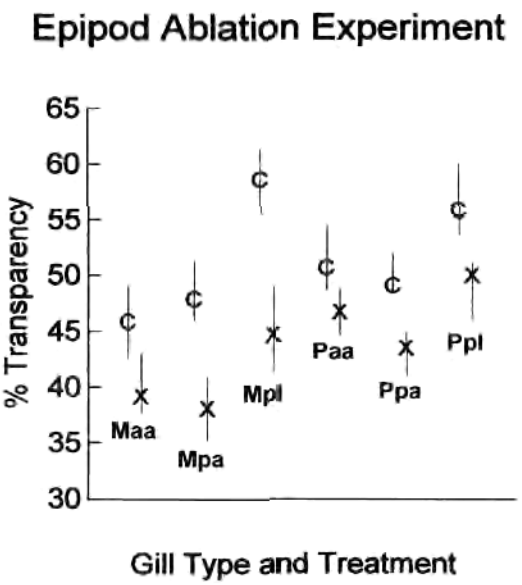

Fig. 10. *Rimapenaeus similis*. Results of epipod-ablation experiment: comparison of particulate fouling on gills from control and experimental branchial chambers, using measures of gill transparency to transmitted light. Medians and 95% confidence limits of percent transparency are given for the gills of the third maxilliped and third pereopod from the control (epipods present) and experimental (epipods removed) branchial chambers (n = 25 individuals). Maa, Mpa, and Mpl, anterior arthrobranchs, posterior arthrobranchs, and pleurobranchs, respectively, of third maxillipeds; Paa, Ppa, and Ppl, anterior arthrobranchs, posterior arthrobranchs, and pleurobranchs, respectively, of third pereopods.

and a natural swamp environment (Bauer, '98). However, the presence of setobranchs in crayfishes did not prevent heavy fouling on control gills by bacteria and a sessile protozoan, *Cothurnia variabilis*. This lack of efficiency in preventing epibiotic fouling was attributed to the nonselective movements of the gill-cleaning setobranch setae (Bauer, '98).

In the present study, treated individuals of *Rimapenaeus similis* were exposed to fouling in a recirculating water system, an environment that can be conducive to heavy epibiotic fouling (Lightner, '83). However, the shrimps were maintained individually at low density, excess food and wastes were removed daily, and, because of an effective filter system, water quality was high. No measurable epibiotic fouling was observed on control or experimental gills of treated shrimps, suggesting that epibiotic fouling pressures were low in the system used and that the ability of the gill-cleaning system of *R. similis* to resist epibiotic fouling was not tested in these experiments. However, the similar mode of operation of this passive gill-cleaning system to that of the crayfish *Procambarus clarkii* suggests the hypothesis, still untested, that the penaeid gill-cleaning system is not effective against epibiotic fouling. It is further hypothesized that, while the gills are kept free of particulate fouling by the combined action of the setiferous epipods and pereopodal exopods, molting is the only escape of *R. similis* from epibiotic fouling.

The evidence that passive gill-cleaning mechanisms are primitive and that cheliped brushing of gills is derived in the Decapoda has been given in Bauer ('81, '89, '98). When cheliped brushing evolves in a group, passive gill-cleaning mechanisms become superfluous and maintenance of them is selected against. Since there are various forms of passive gill cleaning, the question arises: which was or were the mechanisms used by the shrimp-like decapod ancestor (described in Burkenroad, '81)? For the primary gill-cleaning mechanism, the decapod ancestor may have had both setiferous epipods and setobranchs. Borradaile ('07) considered setobranch papillae to be derivatives of the epipods, and Batang and Suzuki ('99) demonstrate the presence of both setiferous epipods and setobranch setae in the passive gill-cleaning system of the mud lobster *Thalassina anomala*. Setiferous epipods or setobranchs are the primary mechanisms in groups in which cheliped brushing has not evolved (Bauer, '81, '89, '98). However, in any decapod group in which passive gill cleaning occurs, these primary mechanisms do not clean the gill filaments on the lateral surfaces of the gills just medial to the gill cover. Since the ancestral decapod, a swimming shrimp-like organism, must have had well-developed pereopodal exopods equipped with plumose (natatory) setae, cleaning of the outer filaments must have been performed by some non-exopodal mechanism, e.g., branchiostegal setae or scaphognathite cleaning setae. In most decapods, pereopodal exopods are absent or reduced and no longer natatory. In *Rimapenaeus similis* and perhaps other penaeoid shrimps, the reduced exopods have been modified into gill-cleaning structures. The absence, presence, and relative importance of gill-cleaning setae on reduced exopods, on the inner side of the gill cover, and fringing the scaphognathite will have to be described in more decapod groups in order to hypothesize primitive and derived states in these characters among the Decapoda.

The pereopodal exopods were shown in this study to be important in cleaning the gill surfaces just medial to the gill cover. It is not known if the exopods of other penaeoid

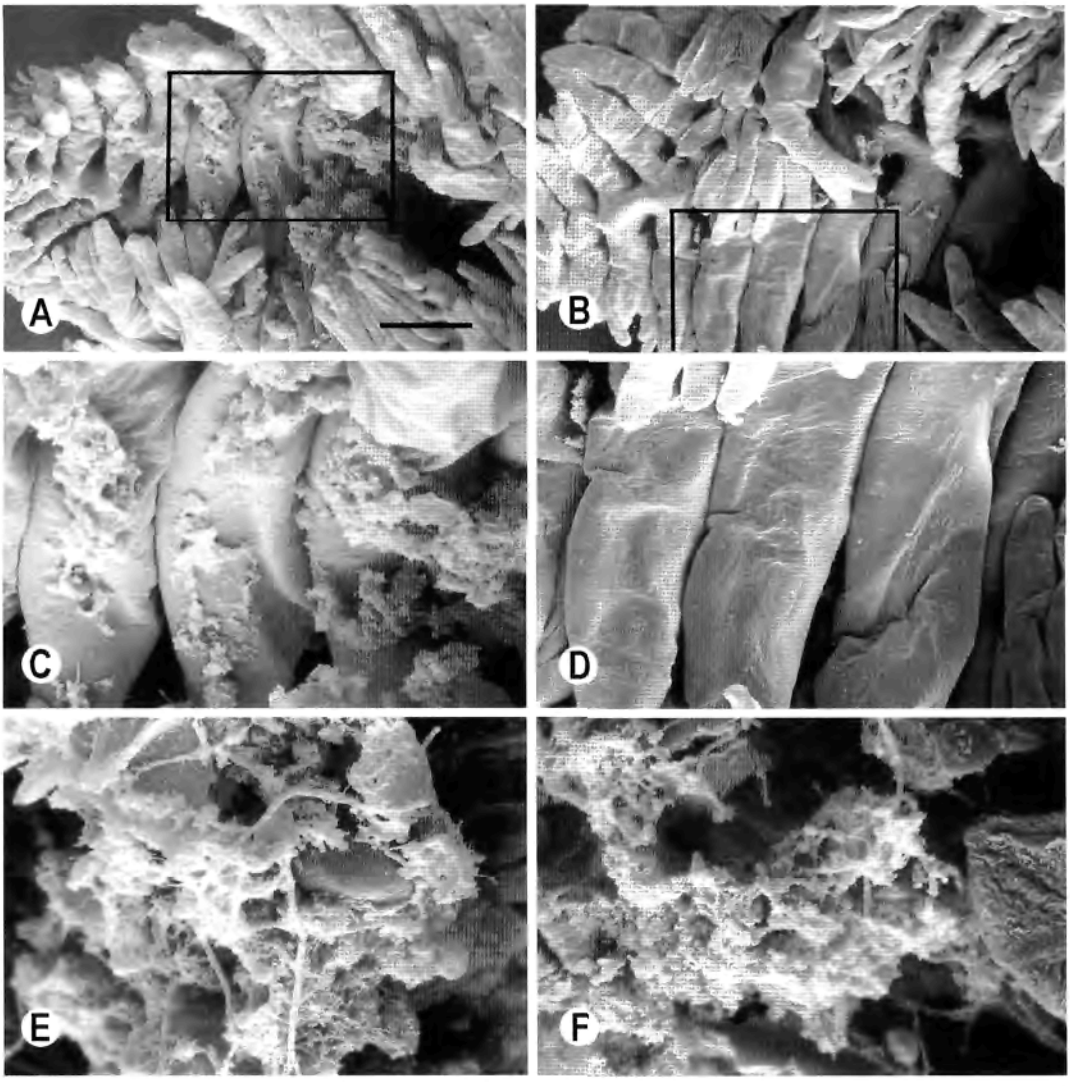

Fig. 11. *Rimapenaeus similis*. Results of the exopod-ablation experiment: comparison of fouling from experimental and control gills of the same specimen. **A:** Lateral side of distal end of anterior arthrobranch, pereopod 2, from experimental branchial chamber, showing particulate fouling (compare with **B**). Box shows area magnified in **C**. **B**: Same gill type and view as **A**, but from control branchial chamber. Note relative absence of fouling (compare with **A**). Box shows area magnified in **D**. **C:** Particulate fouling on filaments in experimental gill (compare with **D**) from boxed area in **A**. **D**: Lack of fouling of filaments of control gill (compare with **C**) from boxed area in **B**. **E,F**: High magnification of particulate fouling on experimental gill shown in **A**. Scale bar = 140 µm in **A** and **B**, 55 µm in **C** and **D**, 9 µm in **E**, and 7 µm in **F**.

genera participate in gill cleaning. Exopods similar to or smaller in size than those of *Rimapenaeus similis* are present on all pereopods in genera of Penaeidae (except *Artemesia*; Pérez Farfante and Kensley, '97) and Solenoceridae. Preliminary observations in *Litopenaeus setiferus* show that the setae on its exopods are multidenticulate like those of *R. similis*; however, those of *Solenocera vioscai* are not. All species of Aristeidae and Benthesicymidae lack pereopodal exopods. Exopods of representative penaeoid species need to be studied to determine if these structures are involved in gill cleaning as in *R. similis*. When they are absent or do not function in gill cleaning, the mechanisms that may replace them, such as branchiostegal, scaphognathite, or other cleaning setae, need to be determined. An array of such characters from penaeoid gen-

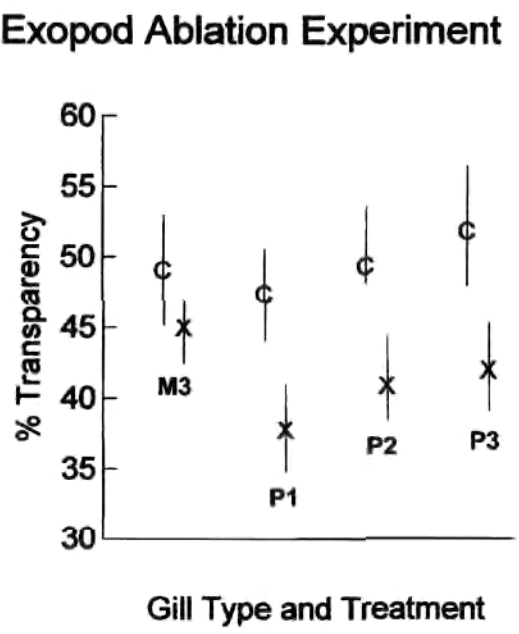

Fig. 12. *Rimapenaeus similis*. Results of the exopod-ablation experiment: comparison of particulate fouling on gills from control and experimental branchial chambers, using measures of gill transparency to transmitted light. Medians and 95% confidence limits are given for percent transparency of the anterior arthrobranchs of the third maxilliped and pereopods 1–3 from the control (exopods present) and experimental (exopods removed) branchial chambers (n = 25 individuals). M3, P1–P3, anterior arthrobranchs of the third maxillipeds and pereopods 1–3, respectively.

era will be extremely useful in studies on the evolution of gill cleaning, as well as in analyses of phylogenetic relationships in the Penaeoidea and other decapod crustaceans.

In penaeoid shrimp, the primary gill-cleaning mechanism, setiferous epipods, is often reduced. In the families Aristeidae, Benthesicymidae, and Solenoceridae, there are epipods on every thoracic limb back to and including the fourth pereopod (branchial formulas given in Pérez Farfante and Kensley, '97). In *Rimapenaeus*, as well as in several other genera in the Penaeidae and all Sicyoniidae, the epipod of the third maxilliped is absent. In *R. similis*, it was observed that the epipod of maxilliped 2, because of its size, greater array of multidenticulate setae relative to the pereopodal epipods, and its oblique positioning in the branchial chamber, was able to take the place of the "missing" epipod of the third maxilliped. This may also be the case in other penaeoid species with the same pattern of epipod number. In *Parapenaeus*, *Parapenaeopsis*, and especially in some species of *Megokris*, *Trachysalambria*, and all species of *Trachypenaeus* (s.s.), the number of pereopodal epipods may be reduced to only one (pereopod 3), with only it and that of maxilliped 2 available for gill-cleaning. Studies are needed to determine if and how that deficiency of basic gill cleaning equipment is compensated for in these taxa.

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