of breast-feeding patterns were incorporated into the analysis. A multiple linear regression with menstrual status as a binary dependent variable was performed using an unweighted least squares analysis. The assumption of constant variance required by this technique was approximately satisfied, the proportion of amenorrheic women at each postpartum interval being generally within the limits of .30 to .70. Since we were not interested in predicting the probability of being amenorrheic but rather in estimating the relation of the independent and dependent variables, the unweighted least squares technique was considered appropriate. This analysis illustrated that when postpartum interval was controlled, maternal age, socioeconomic status (defined by wealth), and supplementary feeding of the infant were correlated with the probability of being amenorrheic; the correlations were statistically significant but the total amount of variance explained was not large ($R^2 = .115$): As has been found in other studies (*9*), older women were more likely to be amenorrheic than young women; richer women and those feeding their infants the better supplements were more likely to have resumed menstruation than the others in the same postpartum interval. With all variables present, maternal nutritional status explained less than 1 percent of the total variance. Current maternal and infant morbidity and infant anthropometric status were not associated with the probability of amenorrhea.

Socioeconomic status (wealth) was found to be unrelated to maternal nutritional status. This surprising finding may be explained by the generally poor sanitary and environmental conditions prevalent throughout villages in Bangladesh and by the low status of women in all socioeconomic ranks. We suspect that the independent effect of socioeconomic status on lactational amenorrhea reflects variations in breast-feeding practices. There is some evidence that as the frequency, intensity, and duration of suckling increases, postpartum amenorrhea is prolonged (*10*). Cultural factors differing between socioeconomic classes may result in variations in breast-feeding patterns.

The association between supplementary feeding and postpartum amenorrhea also supports the hypothesis that breast-feeding patterns affect lactational amenorrhea. Women who supplement their infants' diet with nutritious foods probably breast-feed less often than mothers who do not or who do so less. The decreased frequency of suckling would increase the probability of resuming menstruation. In short, breast-feeding patterns rather than maternal nutritional status may prove to be the determining factor in the prolongation of postpartum amenorrhea. Further research is needed to substantiate this theory.

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Absence of Microorganisms in Crustacean Digestive Tracts

Abstract. *Two marine and one terrestrial wood-boring isopod species and one wood-inhabiting amphipod species maintain a digestive tract free of microorganisms. Digestive tracts examined in toto with the scanning electron microscope were devoid of microorganisms. In contrast, the outer exoskeleton surfaces of these crustaceans support a dense bacterial flora. Observations of the hindgut of termites revealed a diverse gut microflora as expected.*

Gastrointestinal microorganisms have been found in a broad range of metazoans (*1*). These observations have led to the assumption that all animal digestive tracts possess an indigenous microflora (*2*). We report a clear exception to this premise. Selected genera of marine and terrestrial wood-boring isopods and a marine wood-inhabiting amphipod, members of the Crustacea, maintain a gut completely free of microorganisms.

Boring isopods cause extensive damage each year to wood structures in marine coastal zones. Species in the genus *Limnoria* have a cosmopolitan marine distribution bounded only by the approximate geographic constraints of 60° north and south latitude (*3*).

Ray and Julian (*4*), using a light microscope, failed to produce evidence of a microflora in the gut of *Limnoria tripunctata* (*5*). They concluded that these wood-borers produce their own cellulolytic enzymes (*6*). A study of *L. tripunctata* in our laboratory, using the scanning electron microscope, confirmed this observation (*7*).

The analysis reported here involved two marine wood-boring isopods, *L. tripunctata* and *Limnoria lignorum*; a marine wood-inhabiting amphipod, *Chelura terebrans*, which lives within the burrows of *Limnoria* species and feeds on limnorid fecal material; and a terrestrial isopod, *Oniscus asellus*.

Digestive tracts (*8*) were removed whole and intact by dissection in buffered glutaraldehyde under a binocular microscope. The tracts were fixed, dehydrated, dried at the critical point, and coated with gold-palladium for scanning electron microscopy by techniques described elsewhere (*7, 9*). The digestive tracts ranged from 1.5 to 2.0 mm in length.

No microorganisms were found in the entire digestive system of *L. tripunctata*, *L. lignorum*, and *C. terebrans* during repeated observations with the scanning electron microscope (Fig. 1, A to C). Wood particles taken from the digestive tracts shown in Fig. 1 were examined separately under the scanning electron microscope. The gut contents were devoid of microorganisms. These results led us to conduct a similar study on a phylogenetically close relative of *Limnoria* spp. which lives in an entirely different habitat. The wood-inhabiting isopod *O. asellus* is found in rotting logs (*8*). The entire digestive tract of this isopod was found to be devoid of microor-

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ganisms when analyzed with the scanning electron microscope (Fig. 1D).

Concurrent observations showed that the outer exoskeleton surfaces of these crustaceans are heavily colonized by bacteria. The complex surfaces of the pleopods and the pleotelson exhibit particularly dense bacterial attachment. Populations often reach densities of more than five cells per square micrometer on these surfaces (Fig. 1E). The wood surfaces within the burrow are also well colonized by bacteria (*7*).

Termites were analyzed to ensure that our techniques were not removing microorganisms from the digestive tracts observed in our study. Breznak and Pankratz (*10*) demonstrated that, in addition to a protozoan population, the termite *Reticulitermes flavipes* possesses a symbiotic bacterial flora which lines the lumen surface of the hindgut epithelium. Specimens of *R. flavipes* (*8*) were prepared for scanning electron microscopy by procedures identical to those used in the preparation of crustacean digestive tracts. The termite gut was found to be

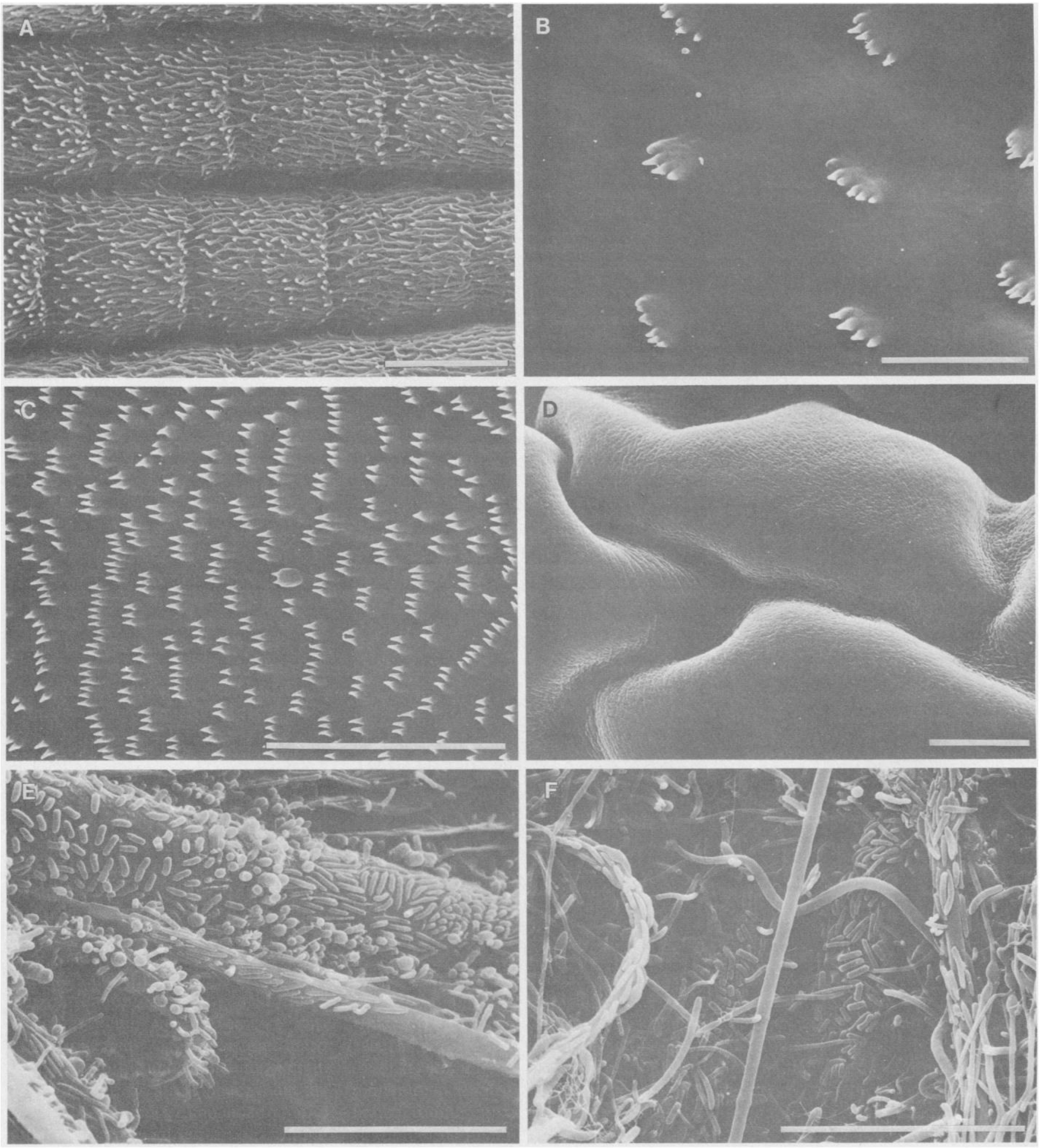

Fig. 1. (A) *Limnoria tripunctata* hindgut showing complex surface devoid of microorganisms. (B) *Limnoria lignorum* midgut showing bacteria-free digestive tract surface. (C) *Chelura terebrans* hindgut illustrating the bacteria-free condition seen throughout the entire digestive tract. (D) *Oniscus asellus* anterior gut surface free of microorganisms. (E). Exoskeleton of *Limnoria lignorum*; pleopod with typical dense colonization by bacteria. (F) *Reticulitermes flavipes* hindgut showing diverse, dense microflora lining the digestive tract surface. All scale bars, 10 μm.

lined with a diverse microbial flora (Fig. 1F). We attempted to confirm the absence of microorganisms in the digestive tracts of *Limnoria* spp. by using conventional microbiological plating techniques. However, the small size of the gut and the extensive microbial colonization of the complex exoskeleton surfaces (Fig. 1E) prevent use of these methods.

Bacteriological studies of the decapod *Homarus vulgaris* (*11*) and the deep-sea scavenging amphipod *Hirondellea* sp. (*12*) recently demonstrated that these crustaceans maintain an indigenous microflora within the stomach and intestine. The mechanisms by which the crustaceans observed in our study maintain a bacteria-free digestive system remain unexplained. It is possible that enzymes produced by these organisms lyse ingested bacteria (*13*). However, no bacterial cell fragments have been observed within the gut. A gut exudate may be toxic to bacteria. This hypothesis is supported by our observation that *L. tripunctata* fecal matter is not readily colonized by bacteria. In contrast, the overlying water supports an abundant microflora. Further research into the antimicrobial processes active in these crustaceans is needed.

The precise microbiological status of the gastrointestinal systems in the overwhelming majority of invertebrates remains to be determined (*14*). Marine wood-boring bivalves and oysters are known to possess an indigenous microflora in their intestinal tracts (*15*). Echinoids have also been found to support a gut microflora (*16*). However, the species of *Limnoria*, *Chelura*, and *Oniscus* observed in our laboratory appear to be the only known metazoans with digestive systems naturally free of microorganisms. The bacteria-free gut, observed as a normal condition in these invertebrates, should provide a model system for research on axenic and gnotobiotic digestive tracts, which has heretofore depended on extraordinary measures to produce artificial sterility. Furthermore, it may provide a new source of antimicrobial agents.

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9. Fixation was in 2 percent glutaraldehyde buffered with 0.025*M* sodium cacodylate in natural seawater filtered at 0.45 μm. Dehydration was accomplished in an acetone–distilled water series (10, 20, 40, 75, 95, and 100 percent acetone). Critical point drying was in liquid CO_2; samples were viewed under an AMR 1000 scanning electron microscope at the Harvard Museum of Comparative Zoology.
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Amino Acid Sequence of the Fc Region of a Canine Immunoglobulin M: Interspecies Homology for the IgM Class

Abstract. *The amino acid structure for the Fc portion of a canine immunoglobulin μ chain was determined. The sequence was compared with those of two human μ chains, and a high degree of interspecies homology was observed. The preservation of primary structure between species is probably reflective of the unique functions associated with the immunoglobulin M class.*

Amino acid sequence analyses of the κ, λ, and γ chain constant regions of immunoglobulin reveal a modest degree of sequence homology when these regions are compared with respect to species (*1–3*). When the amino acid residues at a particular position in immunoglobulin chains are compared, most studies indicate approximately 60 percent preservation of primary structure (*4*). In the variable region, such calculations are more complicated because of V region subgroups. However, where subgroups can be clearly related, the interspecies homology within the variable region also approximates 60 percent. If hypervariable regions are excluded from this comparison, homology in the variable region often approaches 85 to 90 percent (*5, 6*).

Phylogenetic, evolutionary, and immunochemical data suggest that the immunoglobulin M (IgM) class has been more rigidly preserved in evolution than κ, λ, or γ chains (*7*). Studies on the serologic cross-reactivity of the various classes and types of immunoglobulins have repeatedly demonstrated that IgM molecules cross react more extensively than do immunoglobulin G (IgG), immunoglobulin A (IgA), κ, or λ chains (*8*).

We have recently completed the variable region sequences of a canine IgA and a canine IgM (*9*). We now report the complete amino acid sequence of the Fc region of a canine μ chain and compare the sequence with human μ chains.

The monoclonal canine IgM Moo was isolated by plasmapheresis from the serum of a dog with lymphosarcoma. Starch block electrophoresis, ion exchange chromatography, and chain separation techniques were used to determine its primary structure (*9*). Cyanogen bromide peptides were isolated and subjected to extensive amino terminal sequence analysis with an automated Beckman 890C sequencer. Subsequently, appropriate tryptic peptides were isolated and sequenced, some with the aid of Polybrene (*10*). With only a few exceptions, the entire Fc region of the molecule has been sequenced and extensive overlaps were obtained.

Figure 1 presents the amino acid sequence of 272 residues of the Moo Fc region compared to the human Ou (*11*) and Gal (*12*) μ chains. The Fc regions of Ou