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Integumental ultrastructure and color patterns in the iridescent copepods of the family Sapphirinidae (Copepoda: Poecilostomatoida)

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Abstract The ultrastructure of the integument of the sapphirinid copepods was studied by scanning and transmission electron microscopy. Samples were collected between 1991 and 1993 by plankton-net tows from the subtropical and tropical waters of the North Pacific. In all the seven species examined of *Sapphirina* and *Copilia*, a structure with multilayered platelets was found in the epidermal cells of the dorsal integument of the male. Each platelet is a regular hexagonal prism. The platelets form a plate with honeycomb arrangement within each epidermal cell. Just ventral to the dorsal cuticle, 10 to 14 plates are located parallel to each other and to the cuticle. The mean diameter and thickness of the platelets measured between 1.0 and 1.8 μm and 61 and 83 nm, respectively, for the four species. The specific coloration of seven species was examined with reflected and transmitted light. The iridescent color may be explained by the theory of multiple thin-layer interference in some species which are considered to have an ideal laminar structure, but for the other species, mechanisms from non-ideal systems, including pigment-thin layer interaction, may also be involved.

Introduction

The sapphirinid copepods are distributed widely in the epipelagic zone of the tropical and subtropical waters (Lehnhofer 1926, 1929; Sewell 1947; Boxshall 1977; Rajaram and Krishnaswamy 1981, etc.). They are frequently collected together with salps in plankton nets (Hardy 1936; Furuhashi 1966), which suggests a relationship between these animal groups. Heron (1973) observed females of *Sapphirina angusta* feeding on *Thalia democratica* and considered that their mouth appendages were adapted for attachment to the body surface and for eating the tissue of salps. According to a recent phylogenetic analysis on Poecilostomatoida (Ho 1991), Sapphirinidae is a sister taxon of Sabelliphilidae which is known to be associated with zoantharians, polychaetes, bivalves, holothurians and ascidians, but quite far from Corycaeidae, Oncaeidae and Paralubbockiidae which are usually found in plankton samples. These observations suggest that the sapphirinids would be an interesting marine animal group to examine when considering the significance of parasitism and its evolution in pelagic communities.

Another striking characteristic of the sapphirinids is the beautiful iridescence of the male (see Fig. 3 A to E) as described, for example, by Dana (1852). The generic name *Sapphirina* and many other species names have been chosen with respect to their iridescent colors. It has been suggested in general review papers that this iridescence is caused by crystalline reflection from the integument (Davis 1955, p. 43; Nicol 1960, p. 493–494, etc.). Actually, the macrostructure of the integument has been well studied at the light microscope level by earlier workers (Schmidt 1949, and literature therein; Rose and Vaissière 1951), but the ultrastructure of the integument and the direct mechanism of the iridescence are unknown.

The present study examines the ultrastructure of the integument of sapphirinid copepods using transmission (TEM) and scanning electron microscopy (SEM), describes specific color patterns on the basis of light-microscopic observations, and discusses the causal mechanism of the iridescence.

Materials and methods

Copepods of the family Sapphirinidae were collected by plankton-net tows in the eastern subtropical Pacific off the Philippines (July 1991), in the Kuroshio region off southern Japan (December 1992), and in the tropical North Pacific (February 1993) during the cruises of the R. V. "Hakuho Maru" or "Tansei Maru" of the Ocean Research Institute.

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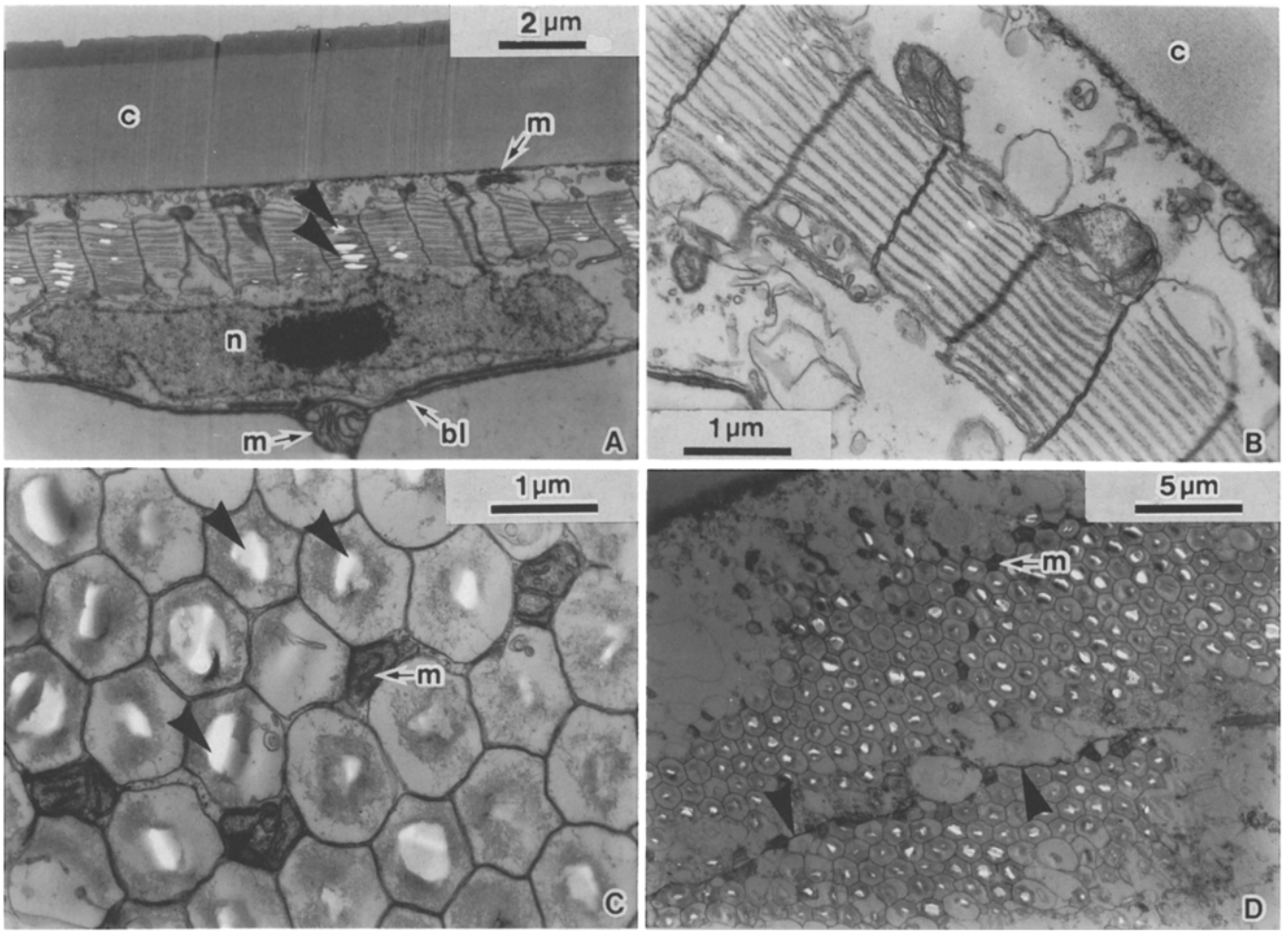

Fig. 1 *Sapphirina opalina*, male. Transmission electron microscopy photographs. (A) Sagittal section of dorsal integument, gaps of different sizes shown (arrowheads); (B) sagittal section of dorsal integument at high magnification, showing multilayered-membrane structure; (C) frontal section of integument, showing honeycomb arrangement of dorso-ventrally oriented membrane and gaps (arrowheads); (D) frontal section of integument, showing honeycomb structure and borders of epidermal cells (arrowheads); bl: basal lamina; c: cuticle; m: mitochondrion; n: nucleus

The internal ultrastructure of the integument was examined with TEM for adult females and males of *Sapphirina opalina* Dana. Live specimens were fixed in 2% glutaraldehyde and 2.5% paraformaldehyde buffered with 0.1 *M* Millonig's phosphate buffer for 2 wk and postfixed in 1% OsO_4 for 2 h, both at 4 °C. The samples were dehydrated through a graded ethanol series from 50 to 100% ethanol and embedded in Epon 812 resin. Thin sections were stained with uranyl acetate and lead citrate and examined with a JEOL 100-CX TEM at an accelerating voltage of 80 kV.

The reflecting platelets (see "Results") were also examined by SEM using the dry-fracturing method (Toda et al. 1989). SEM was necessary since the platelets had been decomposed during TEM fixation, probably as a result of fixation and dehydration, and were missing from sections (see, for example, Nagaishi and Oshima 1992). Adult males of *Sapphirina angusta* Dana, *S. darwinii* Haeckel, *S. gastrica* Giesbrecht, *S. intestinata* Giesbrecht, *S. nigromaculata* Claus, *S. opalina* and *Copilia mirabilis* Dana were examined. Some specimens were kept alive in filtered seawater and brought back to the laboratory, while the others were immediately frozen at –80 °C. The live or frozen specimens were rinsed in distilled water and air-dried at room temperature. The dorsal integument of a dried specimen was removed, attached with the internal surface uppermost to double-sided adhesive tape and mounted on a stub. Another piece of tape was pressed down lightly on the specimens so that the underlying tissue of the dorsal integument was fractured. The tape to which the fragments of tissue adhered was remounted on the same or another stub. This operation was repeated several times until the platelets were exposed. The mounted specimens were coated with gold or platinum and observed with a JEOL JSM-6300F or a Hitachi S-4500 field emission SEM at an accelerating voltage of 5 or 7 kV.

The iridescent colors of live males were examined in seven species (see Table 2) under a stereo microscope in a dark room with white light normally incident to the dorsal plane of the copepod, or with transmitted light. During observations, the copepod was kept in filtered seawater in a small square dish made of microscope slides sealed with silicone grease and pressed softly with a coverslip so that the movement of the copepod was suppressed.

Results

In *Sapphirina opalina*, a multilayered-membrane structure was found in the epidermal cells just ventral to the dorsal cuticle of the male (Fig. 1 A). This structure is distributed continuously over the whole area of the dorsal integument of the prosome and urosome except the caudal rami. It was not found in the ventral integument of either the male or the female, or in the dorsal integument of the female. In

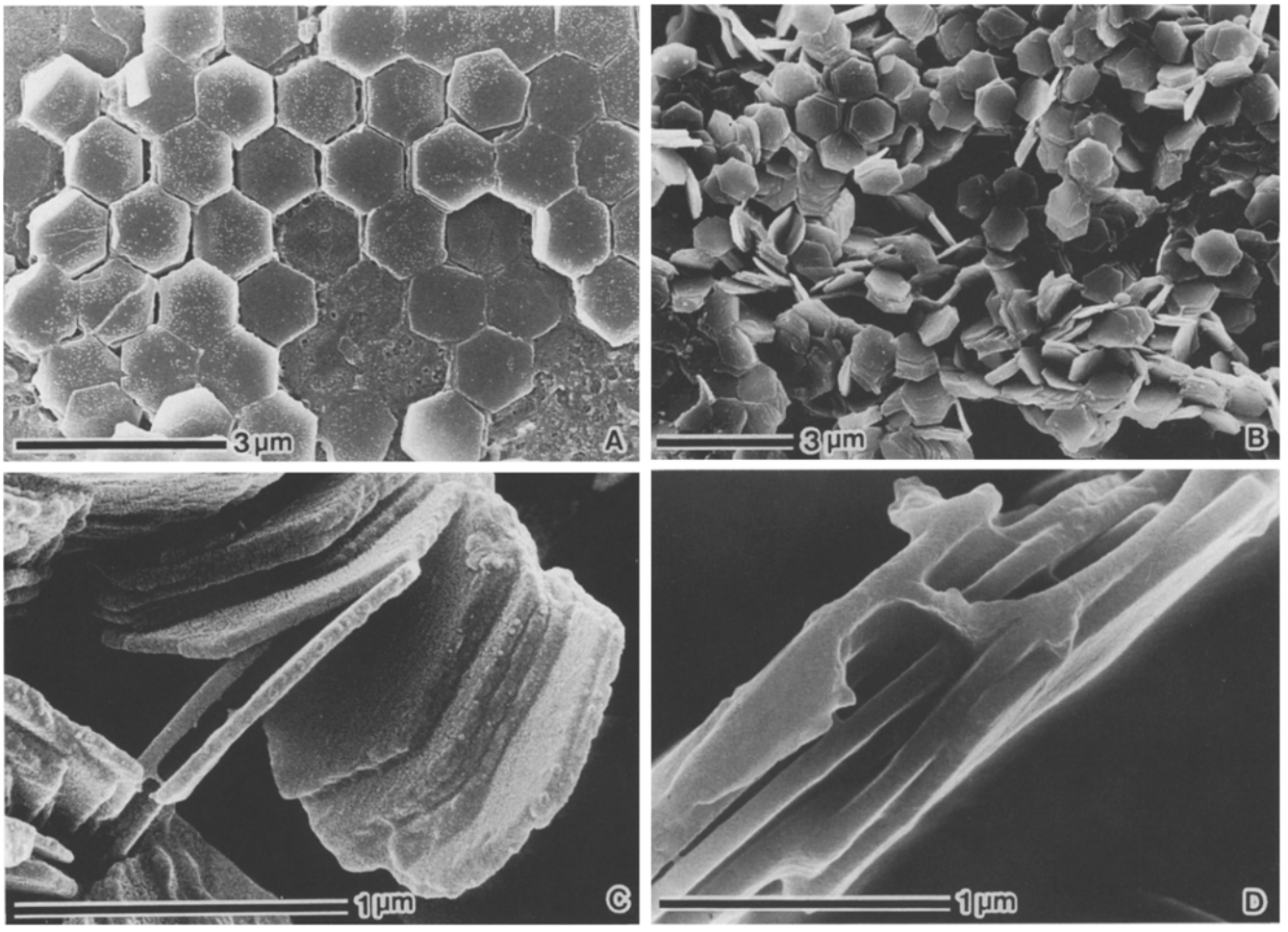

Fig. 2 *Sapphirina* spp., males. Scanning electron microscopy photographs of platelets from dorsal integument. (A) *S. darwinii*, showing honeycomb arrangement; (B) *S. angusta*, showing platelets at different angles, the regular arrangement of which has been disturbed during fracturing; (C) *S. darwinii* at high magnification; (D) *S. nigromaculata*

sagittal and cross-sections, this structure consists of 10 to 14 pairs of closely spaced membranes, lying parallel to each other and to the cuticular plane (Fig. 1 B). These membranes are partitioned by dorso-ventrally oriented membranes. A flattened nucleus is located ventral to the multilayer structure. Mitochondria are located between the cuticle and the multilayer structures, and near the cell membrane (Fig. 1 A). The spaces between the laminated membranes of the thin sections often had gaps of various sizes, suggesting that some hard materials originally present at the gap sites were lost during sectioning (Fig. 1 A). Sections parallel to the cuticular plane show a regular hexagonal structure of the dorso-ventrally oriented membranes which form a honeycomb arrangement. Mitochondria are located near the boundaries of the cells (Fig. 1 C, D). Gaps occur in the inside spaces of the hexagons (Fig. 1 C).

Fig. 2 A to D are SEM images of the reflecting platelets obtained by dry fracturing. In all seven species examined, the platelets are regular hexagonal structures. The size of the platelets is shown in Table 1 for four species in which top (Fig. 2 A) and side views (Fig. 2 C, D) of the platelets, appropriate for the measurement, have been obtained.

Table 1 *Sapphirina* spp., male. Platelet size (mean ± SD) and dominant interference wavelength assuming an ideal multilayer system

Species	Hexagon diameter (µm)	Platelet thickness (nm)	Theoretical wavelength (nm)
Sapphirina angusta	1.01±0.007 (*n*=3)	61±0.5 (*n*=5)	439
S. darwinii	1.29±0.027 (*n*=5)	61±2.2 (*n*=8)	439
S. gastrica	No data	77±7.8 (*n*=7)	555
S. nigromaculata	1.83±0.026 (*n*=4)	83±2.8 (*n*=4)	598

The existence of these hard platelets is complementary to the presence of the gaps in the thin sections as described above. This suggests that each platelet is held in place by a pair of parallel membranes and a hexagonal assemblage of dorso-ventrally oriented membranes, presumably resulting in both the constant distance between the parallel platelets and the regular three-dimensional structure of the multilayer system.

Specific differences in the iridescent colors are shown in Table 2 and Fig. 3 A to E. The reflected colors of the co-

Table 2 *Sapphirina* spp. and *Copilia mirabilis*. Specific color patterns in the males of the sapphirinids, observed with reflected and transmitted light

Species	Color patterns	
	Reflected	Transmitted
Sapphirina angusta	Monotonic dark blue	Yellow, occasionally brown
S. darwinii	Yellow, occasionally purple in lateral margin	No data
S. gastrica	Metallic gold, strong reflection	Gold at pigment sites; other part blue
S. metallina	Metallic gold or silver, strong reflection, rapidly changing blue and red with copepod's movement	Broad spectrum, red, yellow, blue; magenta and violet where cuticles overlap
S. nigromaculata	Metallic gold; urosome strong blue	Light blue; urosome red occasionally
S. opalina	Monotonic violet; occasionally green/orange caudal rami	Yellow, occasionally brown and orange
Copilia mirabilis	Monotonic indigo blue	Monotonic yellow

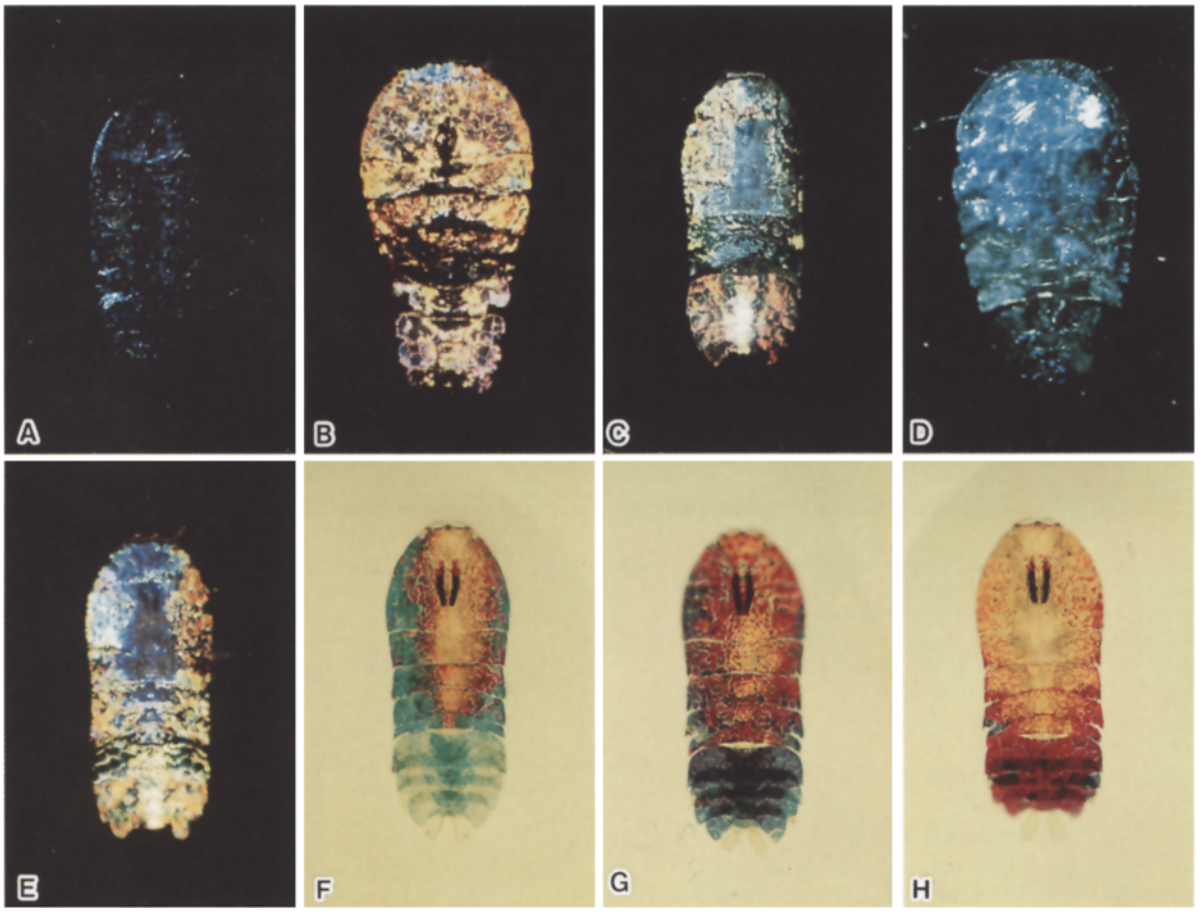

Fig. 3 Sapphirinidae, males. Iridescent colors observed with reflected light (A) to (E) and transmitted light (F) to (H). (A) *Sapphirina angusta*; (B) *S. gastrica*; (C) *S. metallina*; (D) *Copilia mirabilis*; (E) to (H) *S. metallina*, the same individual as in (C), showing color change due to the specimen's slight movement

pepods can be grouped into three types: monotonic color of blue, violet or yellow with a weak reflection (*Sapphirina angusta*, *S. darwinii*, *S. opalina* and *Copilia mirabilis*); strong metallic gold (*S. gastrica* and *S. nigromaculata*); broad spectrum of colors from blue to red which are highly reflected (*S. metallina* Dana). In *S. metallina*, the rapid color change which occurred when the copepod changed orientation was especially marked (Fig. 3 C, E). This was also observed in transmitted light (Fig. 3 F to H). In all species examined, the transmitted colors were complementary to the reflected colors, which is a property of interference systems.

Discussion

According to Schmidt (1949), who reviewed the earlier studies on the iridescence of *Sapphirina* spp., the existence of a "submicroscopic thin layer" (submikroskopische La-

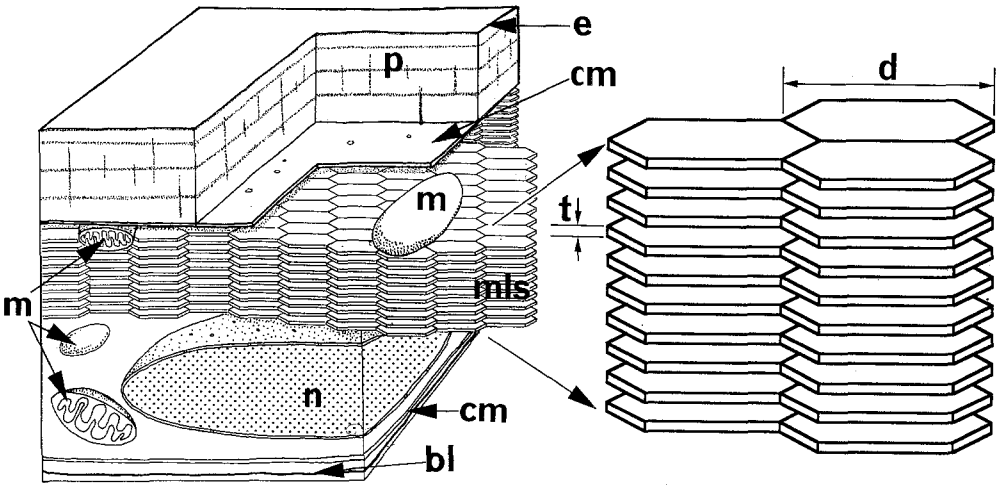

Fig. 4 Sapphirinidae, males. Schematic representation of the structure of the dorsal integument. Membrane structures supporting the platelets not shown. bl: basal lamina; cm: cell membrane; d: platelet diameter; e: epicuticle; m: mitochondrion; mls: multilayer structure; n: nucleus; p: procuticle; t: platelet thickness

mellierung), "hexagonal prisms" (hexagonaler Prismen) or "thin laminae" (Blättchen) had been predicted from light-microscopic observations by several workers (Gegenbaur, Claus, Haeckel, Ambron, Biedermann and Schmidt, all cited by Schmidt 1949), but precise information on the ultrastructure was lacking. Later, Rose and Vaissière (1951), using light-microscopy, described the platelets as consisting of small parallel sticks. However, there have been no further studies since then.

The present observations demonstrate that multilayered, hexagonal platelets exist in the dorsal epidermal cells of the male sapphirinids (Fig. 4). The absence of platelets in the female is consistent with the fact that iridescence is observed only in the male and confirms that this structure is responsible for generating the iridescence. Iridescent, integumental structures with multilayered crystals have been well known in various animal taxa, such as fishes (Denton and Land 1971, etc.), amphibians (Menter et al. 1979) and reptiles (Rohrlich and Porter 1972), but we know of no other literature referring to any such structures in the integument of Crustacea, although multilayer structures have been found in the nauplius eyes of copepods (Land 1984, etc.) including *Sapphirina* sp. itself (Elofsson 1969).

The causal mechanism of structural colors in animals is explained by the theory of multiple thin-layer interference (Land 1966; Huxley 1968; Denton and Land 1971, etc.). When light normally incident on a thin film, which has a thickness t and a refractive index n, is reflected, the wavelength (λ) of the reflected light with maximum constructive interference is equal to 4 nt. The optical properties of the reflecting platelets in *Sapphirina* (species not specified) have been measured by Schmidt (1949) who found that these were strongly double refractive, having a refractive index of 1.79 for rays incident perpendicular to their broad surfaces and a refractive index of 1.55 for rays incident parallel to these surfaces (see also Denton 1970). If we assume a refractive index of 1.8 for the reflecting platelets of all the sapphirinid species, the reflected light will be interfered most constructively at a wavelength $\lambda = 4 \times 1.8\, t$. This equation applies exclusively to ideal multilayer systems, where the optical thickness (nt) of the platelet equals that of the medium between the parallel platelets. The wavelength of maximally reflected light decreases with increased angle of incidence (Land 1966). This is determined by the angle of a particular area of the dorsal integument and by the position of the light source and observer. Thus, an active movement of a copepod will result in a rapid change of the color mosaic, which causes an iridescent appearance.

The color of each species (Table 2) coincides approximately with the descriptions by Dana (1852), though the light conditions of his observations were not defined. The expected wavelength, assuming an ideal multilayer system, is shown in Table 1 for the four species examined in the present study. The existence of three groups with different major color patterns suggests structural differences in their integuments. However, for the hexagonal prisms, there is no consistent relationship between thickness and color pattern, except that the platelets of the species with bright, metallic iridescence are relatively thicker than those of more monotonic species. The iridescent color and the theoretical wavelengths coincide well in *Sapphirina angusta* and *S. gastrica*, which iridesce dark blue and metallic gold, respectively, suggesting that the iridescence of these species is based on an ideal multilayer system. The colors of such species as *S. darwinii* and *S. nigromaculata*, which iridesce yellow and metallic gold, respectively, do not agree well with the expected wavelengths of constructive interference, corresponding to blue (439 nm) and red (598 nm) (Table 1). This suggests that a non-ideal interference system, as discussed in detail by Denton and Land (1971), might be involved in the iridescence of these species. Another possible explanation is that the multilayer-platelet system may interact with a pigment layer lining the epidermal cells (Chae and Nishida unpublished data) or with other pigment cells scattered over the body, such as those known to interact with the iridescent color generation in a fish iridophore (Kasukawa et al. 1987). These interpretations suggest further studies on the integumental ultrastructure of other species not examined here and on the characteristics of platelets including more quantitative, photometric observations.

The fact that only males iridesce, the specific differences in iridescent colors, and the well-developed eyes

(Gregory et al. 1964; Elofsson 1969; Wolken and Florida 1969; Land 1984, etc.) all suggest that iridescence of sapphirinids has an important role in mate finding, presumably by the female (Heron 1973). Unfortunately, we still have only fragmentary information on their biology. For a further understanding of the ecological significance of the iridescence of the sapphirinids, it will be necessary to study their mating behavior, vertical migration and photic environment, as well as the optical characteristics of individuals.

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