CHAPTER 6

Discovery of functional iridescence and its coevolution with eyes in the phylogeny of Ostracoda (Crustacea)

SUMMARY

Highly efficient iridescence due to natural diffraction gratings is reported for the first time in the Crustacea. Iridescence presumably began as an epiphenomenon, but has evolved to include a courtship function in at least some myodocopid Ostracoda (Crustacea). Ostracod iridescence apparently preceded the evolution, and is probably a precursor, of cypridinid (Myodocopida) bioluminescence. By tracing the development of light reception and display, myodocopid evolution, in part at least, is revealed. Therefore, light appears to be a major stimulus to myodocopid evolution. The myodocopid lateral eye probably evolved at a similar point in time as iridescence, possibly to detect iridescence. The graduations by which the ostracod compound eye has developed are suggested. These findings challenge

current theories which demand a single evolution of the compound eye.

1. THE MYODOCOPID FIRST ANTENNA AND IRIDESCENCE

Myodocopids (order Myodocopida) are small (1-32mm) marine, mainly benthic crustaceans, although some are pelagic and all bear well developed swimming appendages. They occur world-wide at all depths. Their bodies are enclosed within a bivalved carapace ("shell"). They currently comprise five extant families (Kornicker 1986). Myodocopids reproduce sexually, although details of copulation are poorly documented, and some cypridinids (Myodocopida) produce bioluminescence as a courtship signal (Morin & Cohen 1991). The first pair of myodocopid limbs, the sensory first antennae, bear numerous halophores (figure 1 a-e) constituting the halothalium (Chapter 5). Halophores are setules with a finely annulate exoskeleton with a thin continuous outer layer, presumably to provide flexibility and thus optimise sensory reception (Chapter 5). However, the discovery of iridescence from these halophores (after observing myodocopids from many angles, whereas they are conventionally studied from lateral views, from which iridescence may not be visible) is reported here; the grooved external surface (figure 1 c) acts as a diffraction grating splitting the incident white light into its component colours (figure 2). This iridescence is truly spectacular, appearing like a neon light even under extremely low incident light levels.

Maximum reflection (of blue light) from the halophores of Azygocypridina lowryi (Myodocopida: Cypridinidae) occurs at two points, 180° apart, on a 360° rotation of the antenna lying on its side. Iridescence is not observed at the bases of certain halophores, where ring construction and, therefore, external grooves do not occur. Stained halophores also produce iridescence and these two observations are important evidence that the diffraction gratings produce the major component of the iridescence. Lesser amounts are the product of other light interference effects such as internal reflection, thin-film reflection and second order spectrum diffraction. The cumulative visual effect of the entire halophores of the ostracod is an "iridescent fan". The first antennae can be withdrawn and hidden within the shell, or protruded anteriorly to become highly visible by its iridescent emission.

2. VARIATION OF CYPRIDINID IRIDESCENCE

Many colours can be reflected from the iridescent fan of A. lowryi (eg. figure 3 e-h). Several more highly derived cypridinids (figure 4 a) reflect only blue (or blue/green) light. The grooves of these gratings may be designed, or spaced, to reflect the light into the direction of a chosen diffraction order, giving rise to a greater proportion of energy in that order (Hutley 1982). Hence certain colours (eg. blue) prevail. The ridge widths in A. lowryi lie in the range of the wavelengths for blue, green and yellow light, but in other more highly derived taxa (figure 4 a) a repeat pattern

comprising a number of grooves (spaced more closely) may be involved. The greater the number of grooves or repeat patterns, the higher the intensity of the reflected light (Hutley 1982). The reflected light may also be strongly polarised at certain angles (eg. Von Frisch 1968). The colour observed from an iridescent fan is fixed when the halophores are stationary, and variable, in ostracods bearing gratings which reflect many colours, or fixed but appearing as flashes to the observer, in ostracods bearing gratings designed to reflect only one colour, when the halophores are moved. Living _A. lowryi_ orientate their first antennae so that principally blue light is reflected towards an observer positioned almost in the path of the incoming light, and subsequently retains this position (figure 3 _c_). Blue light penetrates sea water maximally and, therefore, reaches the greatest depths; some animals can detect light at about 1000m (Denton 1990). _Azygocypridina lowryi_ are red/orange in colour (figure 3 _b_) indicating that some light (eg. blue) must be detected in their environment. However, red light is the first to be absorbed with increasing depth and would not reach the environment of this species where, therefore, it would be invisible (as is the case with many other deep sea crustaceans).

3. FUNCTION OF CYPRIDINID IRIDESCENCE

Light is a major resource to exploit. Darwin (1859) stated that "whenever colour has been modified for some special

purpose, this has been, as far as we can judge, either for direct or indirect protection, or as an attraction between sexes".

Iridescence is functional, at least in certain cypridinids. An undescribed shallow water species in the genus Skogsbergia (Australian Museum registration number P45068) is active during daylight hours (personal observation) and is, therefore, subjected to light conditions. During courtship the male of this species swims to the vicinity of the female, probably after detecting female pheromones (eg. Lowry 1986), and when the male's anterior is visible to the female, within about three carapace lengths, the male displays its iridescent fan (figure 3 i-l). The female becomes sexually receptive and copulation follows (Chapter 7). This courtship function of iridescence increases the ostracod's reproductive potential, ie. it becomes "fitter" in evolutionary terms (Bastock 1967).

4. DEVELOPMENT OF THE CYPRIDINID IRIDESCENT FAN AND THE ROLE OF LIGHT IN CYPRIDINID EVOLUTION

The capacity of the iridescent fan as a light reflector has improved throughout cypridinid evolution. In A. lowryi, as in the majority of cypridinids, the most numerous (often long) halophores are found on the s-seta, formerly the sensory seta (Chapter 5) but they also occur on the long terminal first antennal setae (b-, c-, f- and g-setae), and terminally (long) on the d- and e-setae. In the more highly derived cypridinid genera Siphonostra, Skogsbergia and Paravargula (figure 4 a)

sexual dimorphism of the iridescent fan has become pronounced.
Here halophores typically arise in abundance from bulbous
proximal sections of the f- and g-setae of the male first
antennae. These halophores may be branched to produce a dense
fan, flattened for unidirectional reflectance to reduce
scattering, and exhibit the most efficient diffraction
gratings yet found in ostracods. The grooves have the closest
spacings, exactly the same distance apart throughout the
halophore length. Females of these genera have sparse
iridescent fans with optically less efficient gratings.

The development of the iridescent fan in certain non-
bioluminescent extant cypridinids can be traced, revealing a
possible evolutionary sequence. Beginning with a crude
diffraction grating reflecting light intensely but relatively
sparsely in both sexes, the iridescent fan becomes dense with
very efficient gratings reflecting only blue light
unidirectionally in males.

A phylogenetic tree for certain non-bioluminescent
cypridinids can be constructed using the lateral eye character
(perhaps developing from a flaplike process with possibly dark
sensitive areas, McKenzie 1968, through to a fully developed
compound eye), the bathymetrical range of these taxa (from
deep to shallow seas) and the iridescent fan characters. All
these characters are linked to light adaptation. A cladogram
made using only these light adaptation characters, exactly
matches with a cladogram containing the same non-
bioluminescent cypridinid species made using many
morphological characters but excluding the light adaptation
characters (figure 4). It would seem, therefore, that light

has acted as a major stimulus in the evolution of cypridinid Ostracoda.

5. INDEPENDENT EVOLUTION OF THE MYODOCOPID COMPOUND EYE

It is presumed that Ostracoda are a monophyletic group and that myodocopids branched off a part of the podocopid lineage (McKenzie 1972; Maddocks 1982). Podocopids do not bear lateral (compound) eyes. The Myodocopida further diverged to form the Myodocopina and the Halocyprina; lateral eyes and iridescence exist only in the Myodocopina. Myodocopids first appeared in the Ordovician (McKenzie 1972; Maddocks 1982; Siveter & Vannier 1990), the most primitive of the extant taxa, the Cypridinidae, appeared in the Devonian (Siveter & Vannier 1990). Fossil evidence suggests that myodocopids experienced a shift from shallow benthic to deep pelagic environments during the mid-Silurian (Siveter *et al.* 1991). Halophores, and consequently iridescence, probably developed after this shift to deep water due to selective pressures acting for a more efficient food detection mechanism (less food available). Myodocopid lateral eyes may have similarly developed after the migration to deep water, possibly to detect the iridescence. Subsequently, iridescence may have affected the continuing development of these lateral eyes.

The compound eye of <u>Macrocypridina castanea</u> perceives light optimally in an anteroventral direction, to a distance of about 10 times its carapace length (Land & Nilsson 1990). This distance incorporates the range in which the iridescent

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fan is displayed during courtship. Eyes of marine animals are thought to have evolved to optimise vision towards the surface (ie. towards incoming light); were this true, M. castanea would swim upside-down (Land & Nilsson 1990). However, they do not swim upside-down (Davenport 1990) giving evidence that the ostracod lateral eye evolved to perceive something other than surface light, probably iridescence. The lateral eyes of more primitive myodocopid ostracods (figure 5) also appear to have maximum perception anteroventrally.

The other four extant myodocopid families also express both male iridescence and compound eyes. These families probably evolved after the cypridinids (Azygocypridina, Isocypridina, Gigantocypris and Pseudodoloria) which bear primitive or developing eyes (figure 5). Triadocypris spitzbergensis, known only from the Lower Triassic, appears to be transitional between the myodocopid families Cypridinidae and Cylindroleberididae and bears compound eyes (Weitschat 1983). Therefore, following the evolution of Pseudodoloria (with primitive eyes) between the Devonian and Triassic (355-250 Ma), a major evolutionary radiation within the Myodocopida probably took place, forming the origins of the extant families (other than the Cypridinidae). This hypothesis suggests that the Cypridinidae form two distinct clades: taxa evolving before and after Macrocypridina, or primitive and advanced (highly derived) groups (this is supported by shell size and shape, and muscle scar pattern, and may include extinct taxa).

Advanced cypridinid taxa live in shallow water, probably because they require a high level of light. The advanced

cypridinid taxa are very diverse and are, at least in Australian seas, the most abundant scavengers in shallow (less than about 100m depth) marine environments (J. Lowry, personal communication). The ancestral myodocopids (Siveter *et al.* 1987; Kesling & Ploch 1960), possibly without lateral eyes, appear to have been much less diverse and became extinct. This reflects the tremendous evolutionary advantage of a well-developed visual system (Zucker 1994).

Cypridinid bioluminescence, produced from a luciferin-luciferase-oxygen reaction, is also blue (Morin & Cohen 1991); the cypridinid compound eyes are probably most sensitive to this colour (Huvard 1990), being adapted to iridescence (bioluminescence is currently only known in the more derived cypridinids; Cohen & Morin 1993). Therefore, iridescence is probably a precursor to cypridinid bioluminescence.

If lateral eyes developed independently within the Ostracoda, then evidence for monophyly of the Arthropoda, partly dependent on compound eye evolution (Paulus 1979; Osorio & Bacon 1994), is reduced. The ostracod compound eye is comparable to apposition eyes of other crustaceans in general construction (design alternatives for an organ of optimum visual perception are, however, very limited, Schram 1986) but has several unique features (Huvard 1990), providing evidence that independent ostracod eye evolution is a reasonable hypothesis.

The recent discovery of a <u>Drosophila</u> homolog of the *Pax-6* gene found in mice and humans, suggests that eye morphogenesis is controlled by similar genes in vertebrates and insects (Quiring *et al.* 1994). Location of the *Pax-6* gene requires

study in myodocopid and non-myodocopid ostracods. Expression of the *Pax-6* gene should be tested, going back to embryonic development, to discover whether the eye proteins are encoded by the RNA. Maybe *Pax-6* is only functional in obtaining the basic light receptor cell.

6. DIVERSITY OF IRIDESCENCE

Bioluminescence is a more versatile means of displaying light, since, unlike iridescence, it is not limited by available light. However, the more localized visibility of iridescence may sometimes be advantageous because it is less likely to attract predators. Also, its relatively low energy requirement may make iridescence (structural colours) advantageous over bioluminescence (chemically produced) in certain situations (eg. where incident light is low but still present).

I have found iridescence produced by diffraction gratings in other invertebrate taxa, including spiders (Castianeira sp.) and polychaetes (Sthenelais pettibonae). Functional possibilities also exist in these cases, therefore, iridescence (including that produced by multi-layer reflectors, Land 1972; Chae & Nishida 1994) could be a major behavioural and evolutionary phenomenon in many animals.

ACKNOWLEDGEMENTS

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Figure 1. Scanning electron micrographs. (a-c) *Azygocypridina lowryi*, adult female: (a) whole animal, lateral view, left carapace valve removed (anterior to the left, first antennae arrowed); (b) broken halophores of the s-seta showing ring construction; (c) external margin of a halophore of the s-seta (width of 3 adjacent individual ridges at midpoint along a halophore (w) = 480, 570, 550nm). (d-e) Adult *Skogsbergia* undescribed species, tip of left first antennae: (d) female, lateral view (w = 148, 161, 156nm); (e) male, medial view (w = 110, 110, 110nm); f = f-seta, g = g-seta, s = s-seta (not in view on male) - setules arising from these setae are halophores. (f-i) Diffraction gratings on the surfaces of other iridescent taxa. (f) *Aatolana rapax* (Crustacea: Isopoda), aesthetasc of first antenna. (g) *Pherusa* undescribed species (Polychaeta), branchial filament from head. (h) *Castianeira* species (Arachnida), dorsal surface of male abdomen (iridescence previously thought to be the result of internal structures). (i) Ctenophore "comb" (fused cilia make up the grating). The grooves shown in (f-i) run longitudinally (as opposed to transversely in ostracods) and their spacings are less than the wavelength range for visible light. Scales: (a) = 2.5 mm; (b), (c), (f) - (i) = 2 μm; (d), (e) = 100 μm.
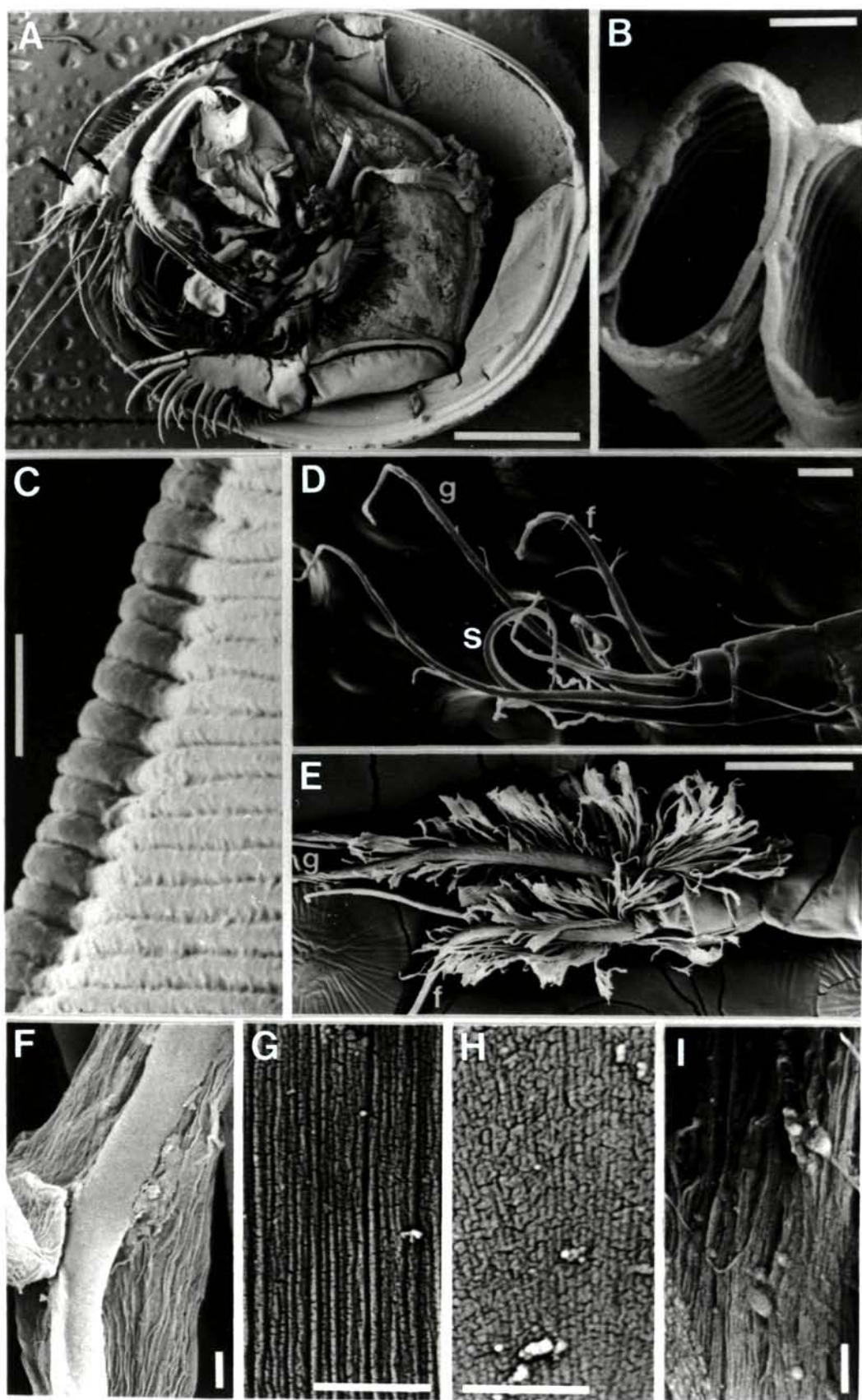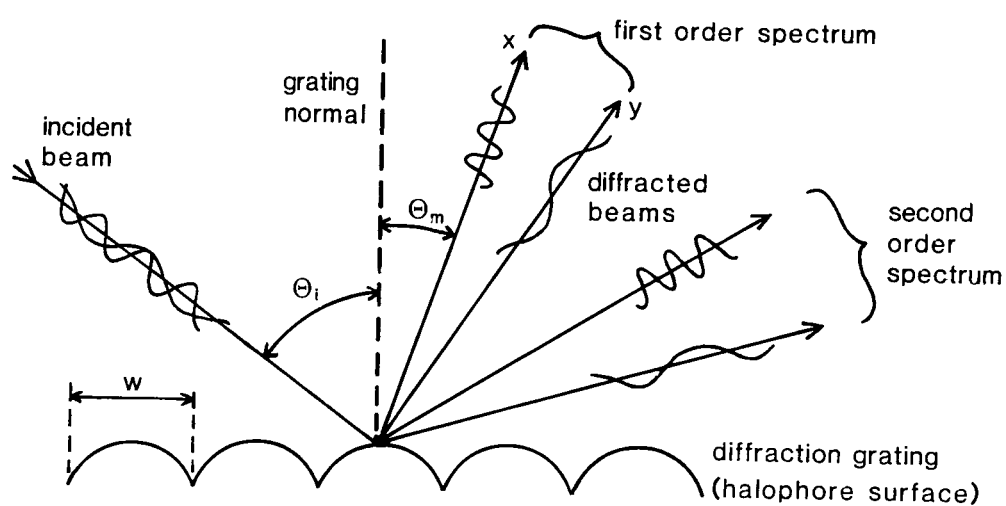
Figure 2. Diffraction grating effect of a halophore surface (w = width of ridges, or the distance between a repeat pattern containing a constant number of ridges; m = order of spectrum reflected). The colour observed depends on the point of observation (eg. the shorter wavelength violet light can be seen at point x, longer wavelength red at point y).

first order spectrum

grating normal

incident beam

Θ_m

Θ_i

x

y

diffracted beams

second order spectrum

w

diffraction grating (halophore surface)

$$\sin \Theta_i - \sin \Theta_m = \frac{m\lambda}{w}$$

Figure 3. (a) Contents of a baited trap set overnight at 300m depth off the N.S.W. coast, Australia (photograph by J. K. Lowry); mainly Azygocypridina lowryi caught. (b-h) Azygocypridina lowryi adult female. (b) Newly preserved specimen; long first antennal setae, but not s-seta, displayed (photograph by Australian Museum). (c) Bases of first antennal setae of a living specimen, showing blue iridescence (mainly from halophores of s-seta), videoed in a large petri-dish under a dissecting microscope, using white fibre-optic illumination (light source is positioned very close to the microscope lens, ie. the angular separation of the light source and microscope lens at any point on the ostracod is small). (d-h) Bases of long first antennal setae (s-seta most obvious) held at various orientations to the incoming white (fibre-optic) light, (d) shows no iridescence (even though bright illumination is used). (i-l) Mating pair of an undescribed Skogsbergia species (collected from Watsons Bay, Sydney, in baited traps set over 24 hours, and subsequently transferred to 20 litres of aerated sea water); male (below) displays iridescent fan (arrowed) to the female in (j-l), (from video recordings of ostracods in a petri-dish under a dissecting microscope, filmed 2 hours after removal from trap; blue colour of iridescent fan has faded during the photographic process); first viewing of a myodocopid mating. Male swims from position in (i) to position in (j); copulation has just ceased and iridescent fan is being withdrawn in (l). Scales: (a) = 10 cm; (b) = 5 mm; (c), (i)-(l) = 1 mm; (d)-(h) = 0.5 mm.
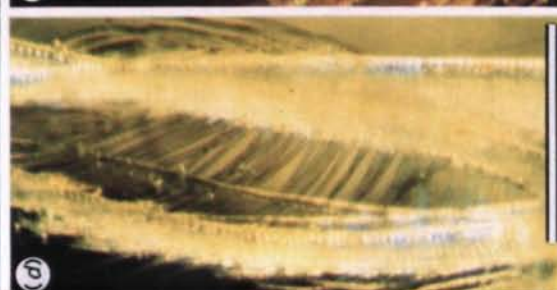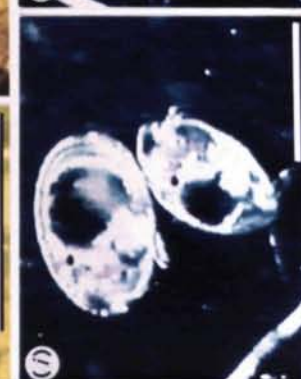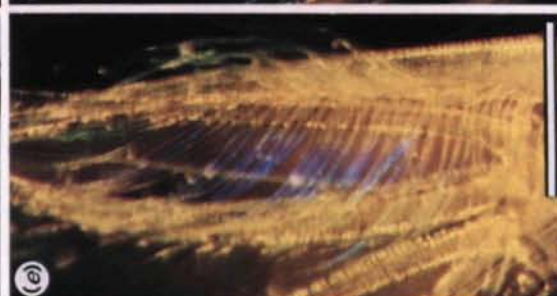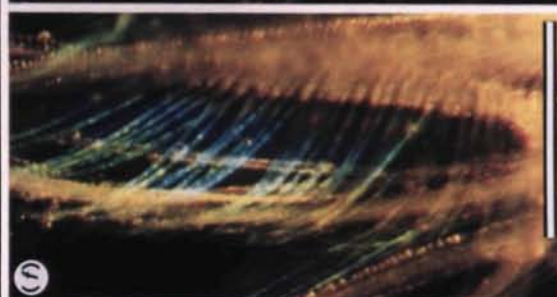
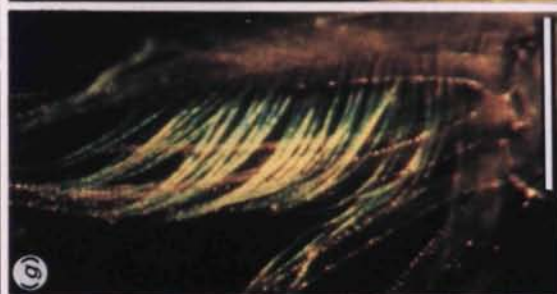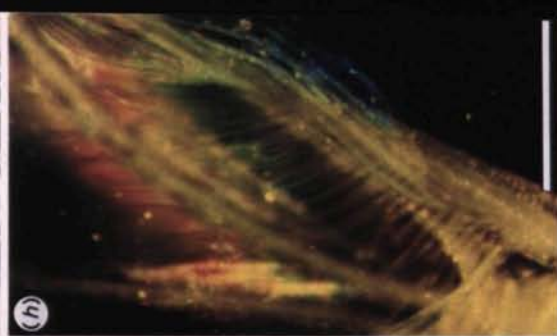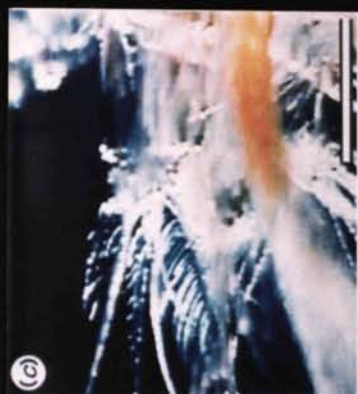Figure 4. (a) Cladogram of certain extant non-bioluminescent cypridinids made using important morphological characters (excluding light-adaptation characters). (b) cladogram of the same non-bioluminescent cypridinids using light adaptation characters only. Both cladograms were produced using CLADOS and show the same phylogenetic relationships. Other taxa may lie at intermediate stages of these cladograms.

(a)

Azygocypridina

Gigantocypris

5	9	11	19	20	22	25
0>1	0>1	0>1	0>1	0>1	0>1	0>1

Pseudodoloria

6	16
0>1	0>2

1	2	3	7	14	15	18	20	25
0>1	0>1	0>1	0>1	0>1	0>2	0>1	1>2	1>2

Macrocypridina

3	9	17
1>2	1>2	0>1

Paradoloria

4	5	8	9	16	23
0>1	1>2	0>1	2>3	0>1	0>1

Doloria

15	16	23
2>1	1>2	1>0

6
0>1

Siphonostra

1	12	13	17	18	21	24
1>2	0>1	0>1	1>2	1>2	0>1	0>1

Skogsbergia

16
1>2

10	15
0>1	2>3

Paravargula

(b)

Azygocypridina

Gigantocypris

1	8
0>1	0>1

Pseudodoloria

3
0>1

Macrocypridina

4	8
0>1	1>2

Paradoloria

4	5
1>2	0>1

Doloria

6
0>1

Siphonostra

2	8
0>1	2>3

Skogsbergia

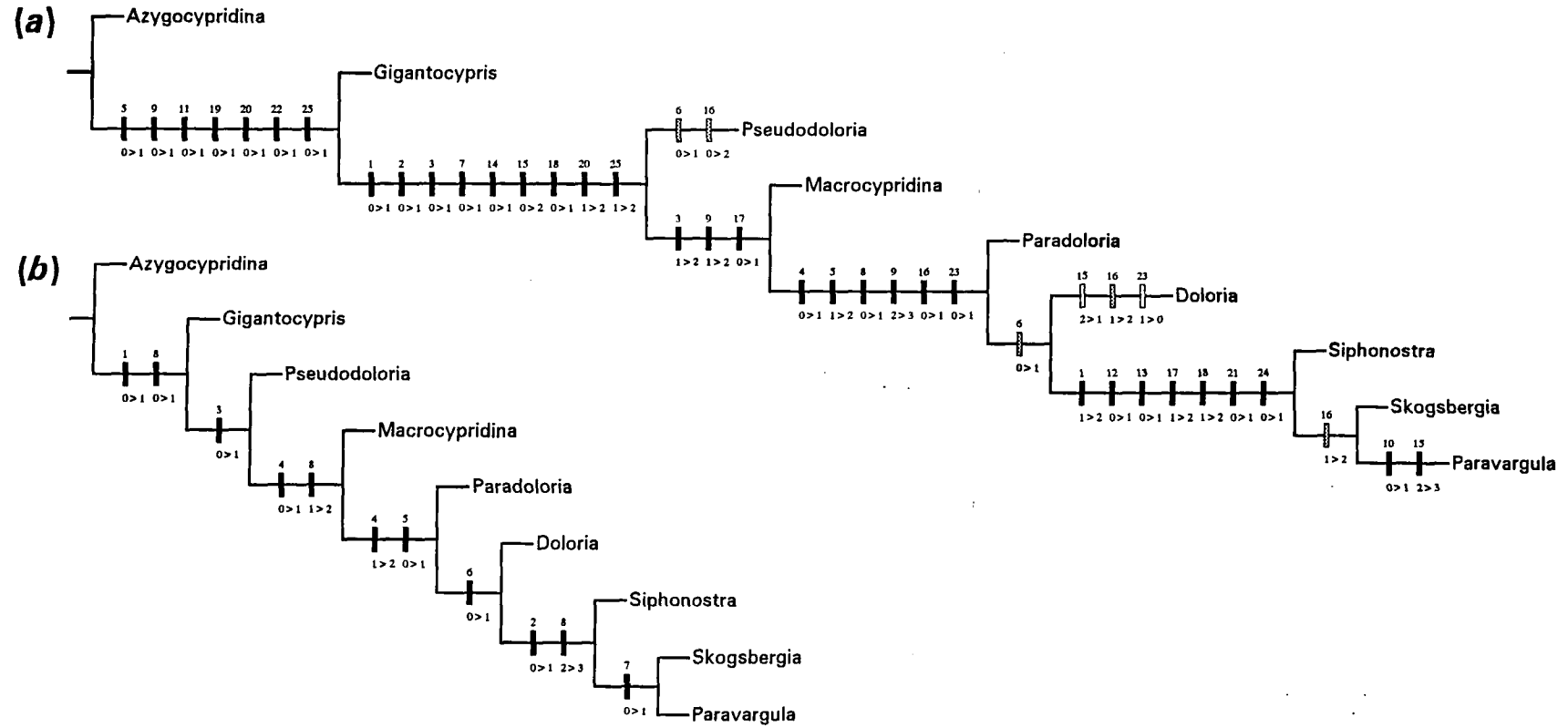| 7 |
|---|
| 0>1 |

Paravargula

Figure 5. Possible means of derivation of the myodocopin compound eye. Anterior of ostracod is to the right, dorsal is above. Dashed arrow represents direction of optimum vision (appears similar for all taxa). The genus _Isocypridina_ bears a similar "eye" to _Azygocypridina_ (Kornicker 1975). Taxa illustrated have relatively primitive iridescent fans. _Azygocypridina lowryi_ after McKenzie (1968), _P. plax_ after Kornicker (1994), _M. castanea_ after Land & Nilsson (1990).

Azygocypridina
lowryi

Gigantocypris
sp.

Pseudodoloria
plax

Macrocypridina
castanea
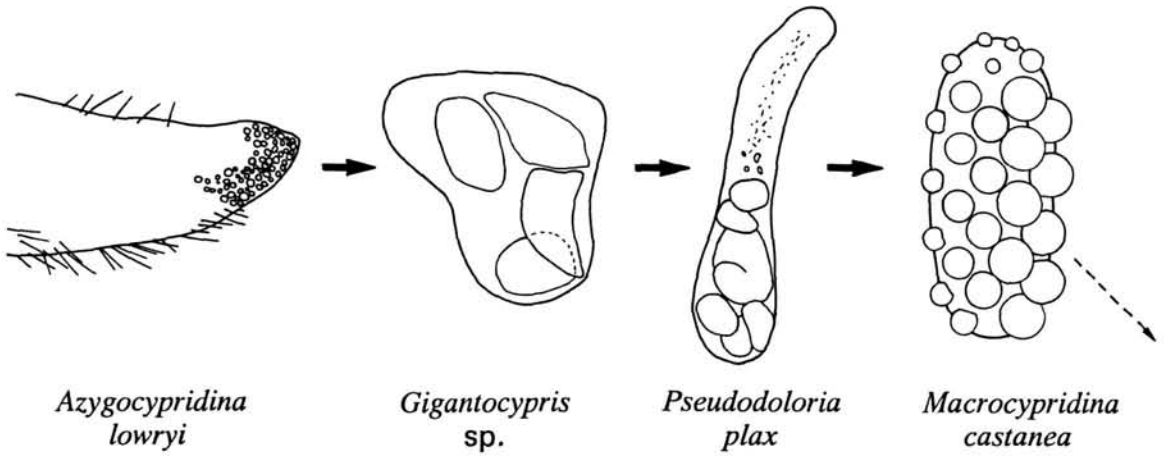
CHAPTER 7

Mating behaviour in myodocopin ostracods (Crustacea): results from video recordings of a highly iridescent species of cypridinid

ABSTRACT

Mating in a myodocopin ostracod (Crustacea) has been captured on video for the first time. The first reliable account of mating in a myodocopin is presented herein for an undescribed species of *Skogsbergia* (Cypridinidae). After initial courtship the male and female ostracods, with ventral margins adjacent and anterior ends directly opposite, join their mandibular claws and furcae. Both furcae are pushed in a posterior direction until the ventral margins of the carapace meet. In this position, with genitalia directly opposite, copulation presumably occurs. This mating procedure lasts for 5 seconds. The male and female second antennae are used to steady the ostracods during mating.

INTRODUCTION

Myodocopin ostracods are diverse marine crustaceans occurring world-wide at all depths. Although myodocopins may be extremely abundant (J.K. Lowry, personal communication), their generally small size (often less than 3 mm in length) has resulted in a lack of behavioural studies, and in particular the copulation process is poorly documented (Cohen & Morin, 1990). The Myodocopina comprise five extant families (Cypridinidae, Cylindroleberididae, Philomedidae, Sarsiellidae and Rutidermatidae); the Cypridinidae are considered to be the most primitive, possibly originating in the Devonian (McKenzie, 1968) or Silurian (Siveter & Vannier, 1990).

All myodocopins reproduce sexually (Cohen & Morin, 1990). The morphology of the myodocopin copulatory "limb" has been documented (Cohen & Morin, 1990; Cohen & Morin, 1993), and recently courtship behaviour has been observed using video recordings in bioluminescent (Morin, 1986; Cohen & Morin, 1993), and non-bioluminescent (Chapter 6), cypridinid species. Some bioluminescent cypridinids use luminescent signalling during courtship; males produce species-specific patterns of flashes in the water column to attract females (Morin, 1986; Morin & Cohen, 1991). Also, some, probably primitive, cypridinids bear clasper-like endopodites on the male second antennae, which could be used for grasping the female during mating (Cohen & Morin, 1990). This requires confirmation using video recordings.

Two accounts of myodocopin copulation have been reported. In 1914 the cypridinid *Vargula hilgendorfii* (Müller, 1890) was

observed mating in a Petri dish (Okada & Kato, 1949). The male clasped the female using the first antennae while swimming with the natatory second antennae. After 30 to 60 minutes they rested on their lateral sides, with ventral margins touching and heads facing in opposite directions; the penis (lying between the paired copulatory "limbs") was protruded into the female carapace and the spermatophore was transferred. This copulation lasted more than 30 minutes (Okada & Kato, 1949). A second account of cypridinids mating was made on *Cypridina dentata* (Mueller, 1906) in an aquarium (Daniel & Jothinayagam, 1979). In this case, the male spread its valves and positioned itself on the posterodorsal part of the female carapace, clasping the edges of the female carapace with the palps of the first thoracic legs (unclear whether these are the fifth or sixth limbs; Cohen & Morin, 1990), and inserted its copulatory apparatus into the female genital opening (Daniel & Jothinayagam, 1979). However, both the fifth and sixth limbs are short with no distinct palps (Cohen & Morin, 1990). During copulation in *C. dentata*, accomplished in minutes, both the male and female luminesced (Daniel & Jothinayagam, 1979). These two accounts describe completely different mating behaviours. Due to the small size of most ostracods, and enclosure of their limbs sometimes within a partly opaque carapace, video recordings would be effective in documenting myodocopins mating with accuracy. Two sarsiellids (Myodocopina) were seen united for only a few seconds (Morin & Cohen, 1991). However, this brief mating, which occurred during sorting of perhaps 100 varied live myodocopids, was too rapid for careful and detailed observation, and the two

sarsiellids immediately became mixed with the rest and were thus not identifiable (A.C. Cohen, personal communication).

Myodocopin copulation probably takes place in the water column. In the philomedid *Philomedes globosus* (Lilljeborg, 1853) the females rise in the water to mate with the males, then bite off the ends of the natatory setae of their second antennae and return to the benthos (Müller, 1898). Indirect evidence suggests that the female natatory setae become broken around the time of egg laying (Kornicker, 1975). However, after losing its natatory setae during its first batch of eggs, the female of *P. globosus* may undergo a second fertilization using sperm that had remained in the spermatheca from the initial impregnation (Skogsberg, 1920; Kornicker, 1975).

MATERIALS AND METHODS

A baited trap consisting of a large jar with a funnel at the entrance, with overlying 4 mm mesh (to prevent large gastropods entering and blocking the funnel), was set for a 24 hour period within Watsons Bay, Port Jackson, N.S.W., Australia (33° 50.85' S, 151° 16.80' E). This was at a depth of 4 m, on a sand and gravel substrate. A smaller jar containing a pilchard bait, with the entrance covered by a 0.5 mm mesh to prevent the prospective trapped ostracods from feeding, was placed inside the large jar. About a hundred specimens of an undescribed species of *Skogsbergia* (Cypridinidae) were caught and immediately transported to the

laboratory in a bucket of sea water (30 cm depth), containing an aerator, for behavioural observations and video recordings (originally intended for feeding analyses).

However, two individual ostracods were observed united within the water column, near the surface. These were transferred to a large Petri dish containing sea water (about 2 cm depth), and videoed using an Olympus SZH stereomicroscope connected to a Panasonic F10 video camera. Mating was recorded on video at midday, and subsequently analyzed frame by frame (eg. Figure 1).

Three male, and one female, specimens of *Skogsbergia* sp. (Australian Museum registration number P45068), were fixed in 5% formaldehyde then preserved in 70% ethanol. The right carapace valves of these specimens were removed. The right limbs were also removed from one of the males. Specimens were cleaned using five half-second exposures to ultrasound, critical point dried using a Bio Rad CPD 750, then coated with gold. The primary and secondary structures used during mating, along with their relative positions, were examined using a Cambridge Instruments S120 scanning electron microscope.

RESULTS

The female *Skogsbergia* came to rest on its lateral side, while the male continued to swim around the Petri dish using its second antennae in an oar-like fashion (as described in Vannier & Abe, 1993). Once within three carapace lengths of the female, and facing in the same direction, the male

displayed its first antennae and, consequently, iridescent fan, anteriorly (striking blue light observed; Chapter 6). The male further approached the female from behind, until the two ventral carapace margins became juxtaposed (Figure 2A). When directly opposite, ventral to ventral and facing in the same direction, the male and female mandibular palps and furcae extended through the aperture of their opened carapaces. The female first antennae were also displayed anteriorly at this point. The mandibular claws and furcae of the male ostracod joined with those of the female (Figure 2B). The female extended the exopodites of its second antennae through the incisure of its carapace, and used them in an oar-like fashion to steady itself against the male. The male used its second antennae (already extended through the carapace aperture) similarly.

With mandibular claws and furcae still joined, and carapaces in the same relative positions, the furcae were thrust posteriorly so that both bodies were fully stretched and the ventral carapace margins made contact (Figure 2C). Due to the striated, and slightly blurred, images produced by the video monitor (eg. Figure 1), the male copulatory apparatus was not clearly observed to enter the female carapace. However, copulation presumably took place in this position, with male copulatory apparatus and female sperm receptacle directly opposite, within reach and without impediments (eg. in Figure 3A the furca is obstructing the extension of the copulatory "limbs", but in Figures 3 B and C the reproductive apparatus have no ventral impediments). It took five seconds from the initial display of the male iridescent fan to the

probable transfer of sperm.

Immediately following copulation, the male and female ostracods withdrew their first and second antennae, mandibular palps, and furcae, into their carapace cavities. Both ostracods rested with their ventral carapace margins still making contact for 30 seconds, although during this time both carapaces were closed (Figure 2D). Then the male swam away using its second antennae.

DISCUSSION

During the filming of this mating procedure, the ostracods were restrained. For example, they were originally observed attempting mating in the water column of the bucket, yet were filmed lying on their lateral sides. Unnatural light and temperature conditions were also probably present. The time of day when species of *Skogsbergia* mate is unknown; I have observed this species to be actively feeding between 1600 and 2200 hours (in light and dark conditions). However, the basic physical procedure reported in this study probably shows little variation to the natural mating procedure because of the detailed, and species-specific, morphology/ultrastructure of the primary and secondary reproductive apparatus of *Skogsbergia* (as with all myodocopins).

The species of *Skogsbergia* used in this study appears to use iridescence as a major feature of courtship. The male's iridescent emission apparently induces the female to become sexually receptive. However, pheromones may also play a role

in the male's initial detection of a female (Lowry, 1986).

The apparatus observed as having a role in mating, ie. the copulatory "limb" (Figure 3D), furcae (Figure 3E), first antennae (Figure 3F), second antennae, mandibles, and presumably the compound eyes, are all often sexually dimorphic within the Myodocopina. This provides further evidence towards their proposed functions during reproduction. The male and female mandibular claws are capable of interlocking with each other due to the lateral separation of the claws (see Vannier & Abe, 1993; Figure 5B).

The sixth limb lies in the vicinity of the genitalia in both male and female myodocopins. Although this limb exhibits little sexual dimorphism, its function is unknown. It is possible that the myodocopin sixth limb has a function during reproduction, but this would be difficult to determine because it is relatively small and transparent, and is always enclosed within the carapace (it could not be observed with clarity during this study). Secondary sexual dimorphism also occurs in the fourth and fifth limbs of many myodocopins (Kornicker, 1981), and this may be related to copulation, female brooding, and/or differences in diets (Cohen & Morin, 1990). Other sexually dimorphic characters, such as carapace size and shape, and the seventh limb, may relate to female brooding (Cohen & Morin, 1990). The "immense" paired copulatory or eighth "limbs" of male myodocopins (Cohen & Morin, 1990) (Figure 3D), with the "penis" in between, presumably play an important role in the copulatory process, details of which require further study.

Male cypridinids bear large and small "suckers" on their

first antennae (Figure 3G). These are believed to be used to grasp the female during courtship (Okada & Kato, 1949), but during this study the male first antennae did not appear to make contact with the female. Also, the numerous halophores surrounding the "suckers" of the males of species of *Skogsbergia* (Figure 3F) may prevent (by obstruction) any possible suctorial function. The functional morphology of these male "suckers" should be studied to test whether they are mechanically viable as such. Maybe the large cypridinid "suckers" could be compared to the morphologically similar calceoli present on the antennae of gammaridean amphipods (Crustacea), thought to act as sensory receptors (Lincoln & Hurley, 1981). Fast atom etching (which removes surface layers; Lincoln, 1985) could be employed for a more precise comparison.

The family Cypridinidae contains bioluminescent and non-bioluminescent taxa. The undescribed species of *Skogsbergia* used in this study is non-bioluminescent, but males are highly iridescent. They have dense iridescent fans (Figure 3F) comprised of flattened halophores (mainly on the f- and g-setae of the first antennae) with closely and regularly spaced external grooves (Chapter 6). This morphology creates very efficient diffraction gratings, reflecting blue light. Females of species of *Skogsbergia* have sparse iridescent fans consisting of less efficient diffraction gratings (Chapter 6). The males of most cypridinids, including non-bioluminescent taxa, bear diffraction gratings which are less efficient than those of species of *Skogsbergia* (Chapter 6). Therefore, the precopulatory or courtship behaviour exhibited by the species

of *Skogsbergia* in this study (ie. the use of iridescence), may be different to that of other cypridinids (although at least most, if not all, cypridinids exhibit iridescence to some extent). The four other extant myodocopin families exhibit well developed iridescent fans in males only, and may utilize iridescence in a similar manner to species of *Skogsbergia*. Therefore, courtship and copulatory behaviour may vary within the Myodocopina, or even the Cypridinidae.

The Myodocopina contains the only ostracods to bear compound eyes, and evidence now suggests that these eyes are important during mating, whether used for the detection of bioluminescence or iridescence. The compound eyes of the cypridinid ostracod *Macrocypridina castanea* (Brady, 1897) may only detect an object as large as itself within a distance of 10 body lengths (Land & Nilsson, 1990). Therefore, a role in mating may be the primary function of the cypridinid compound eye.

Mating in deep-sea (where there is little or no light available for iridescence to function) and planktonic (in relatively high light regimes) myodocopins should particularly be considered for further study of mating. Furthermore, nothing is known of mating within the Halocyprina, bearing often very morphologically different reproductive systems to those of the myodocopins (Wingstrand, 1988; Kornicker & Iliffe, 1989).

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Figure 1. Frame from the video recording of *Skogsbergia* sp. mating. Male is above; female below. Copulation has presumably just ceased and the male iridescent fan (i, reflecting blue light), is in the process of being withdrawn. Mandibular claws (m) are interlocked. Scale = 0.5 mm.
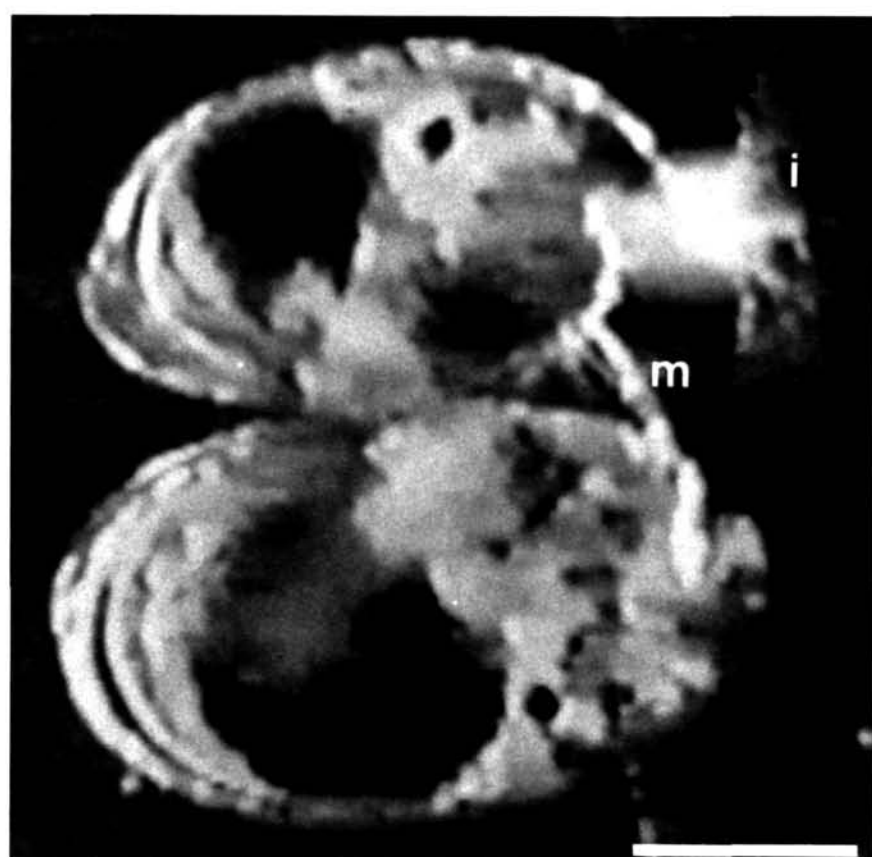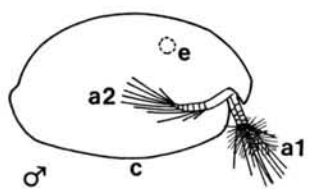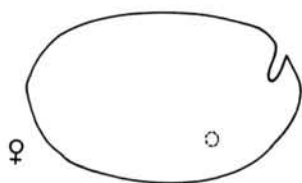
Figure 2. Mating sequence of *Skogsbergia* sp., male above. Copulation is presumably occurring in C. (a1, first antennae; a2, second antennae; e, compound eye; c, carapace; m, mandibular palps; f, furca).
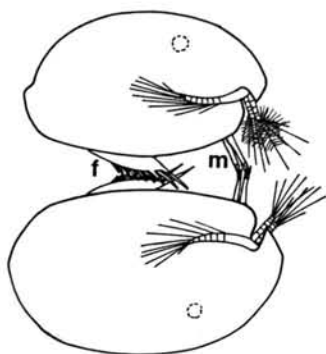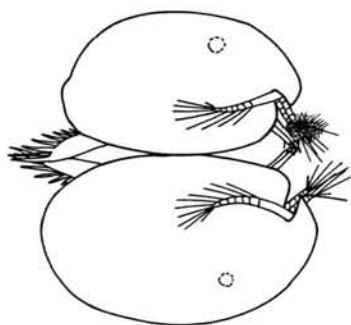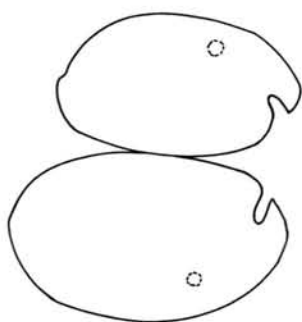
A

B

C

D

Time
(seconds)

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e
a2
a1
c

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f
m

0

1

2

3

4

5

35

Figure 3. Scanning electron micrographs of *Skogsbergia* sp., adult specimens, right carapace valves removed. (A) Male; furca in resting position (anterior is to the right, dorsal above). (B) Male; furca is extended (body is stretched), although it can extend further still; eighth limb is twisted and lying perpendicular to the body. (C) Female; stretched position, although again the furca can extend further still. (D) Male copulatory "limb", lateral view ("penis" is lying hidden between two lobe-like structures). (E) Male furca, anterolateral view. (F) Terminal part of the male first antenna. (G) Large "sucker" of the b-seta of the male first antenna. (a1, first antenna; a2, second antenna; m, mandibular claws; c, male copulatory "limb"; f, furca; d, dorsal body wall; g, opening of the female genitalia; h, halophores). Scales: (A)-(C) = 0.5 mm; (D) and (F) = 0.1 mm; (E) = 0.2 mm; (G) = 10 μm.
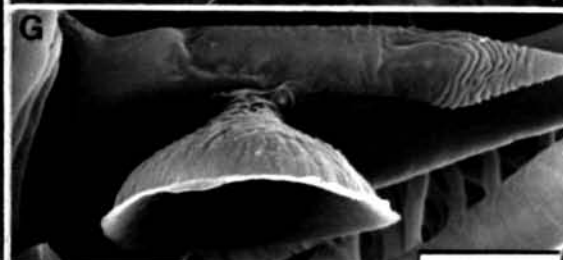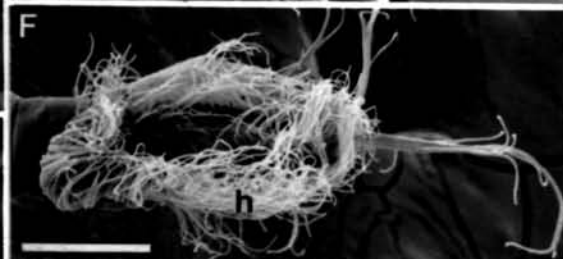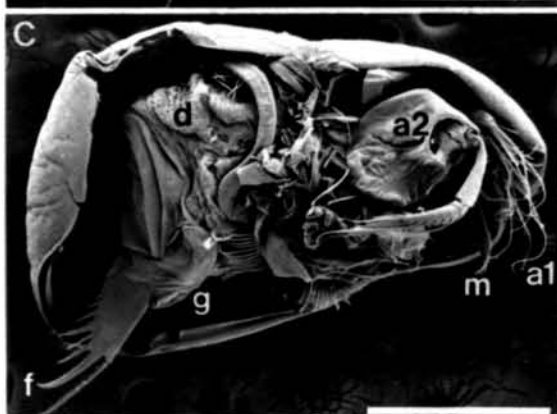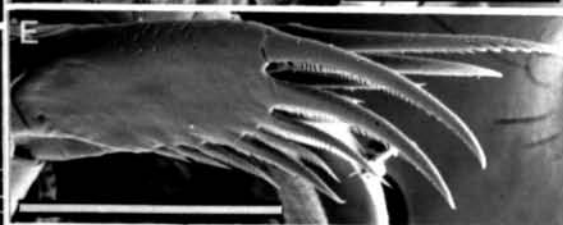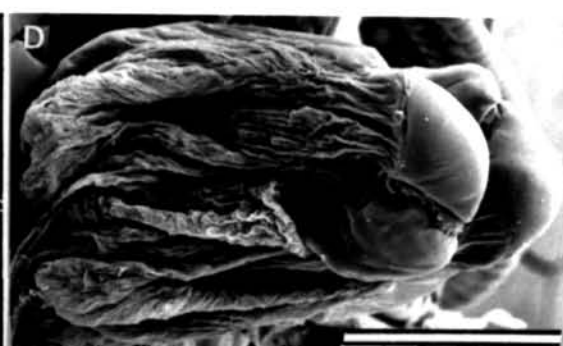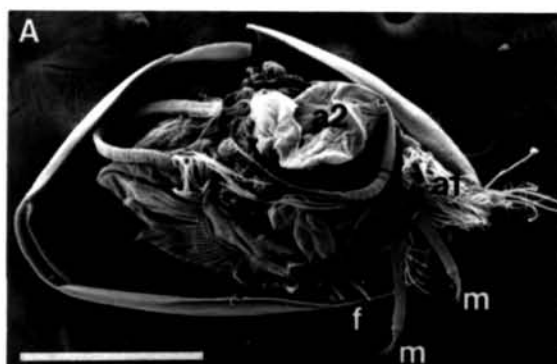
CHAPTER 8

Iridescence: an underestimated phenomenon in crustaceans, and its potential importance in behavioural recognition in marine environments

(Prepared for Philosophical Transactions of the Royal Society of London: Biological Sciences)

SUMMARY

Iridescence, although known to be important in terrestrial ecosystems, has never been considered as a common functional phenomenon in the sea. This study reports that iridescence occurs in many marine crustaceans. Crustacean iridescence may result from multilayer reflectors in the integument, or from diffraction gratings on setae, setules, the carapace selvage, or numerous closely spaced setules may themselves constitute the grating ridges. Iridescence requires incident light, but its employment expends little or no energy. The reflected light is transmitted furthest vertically downwards in the water column.

Crustacean iridescence may function in conspecific

recognition, for example in a reproduction role, or to provide warning (aposematic) colour. I suggest that whenever iridescence is visible externally in the iridescent animal's marine environment, where light is present, the iridescence is probably functional. As such, there may be a relationship between iridescence and the evolution of compound eyes. In particularly well lit environments, where the visual sense of cohabitants is often relatively acute, crustacean iridescence is usually concealed when it is not required. Iridescence may be the most efficient means of light/colour display in low light regimes, where light continues to be a major stimulus for marine animals.

1. INTRODUCTION

Iridescence is the separation of white light by physical structures, resulting in the dispersal of different wavelengths of light (ie. "colours") at different angles. The renowned iridescent effects of terrestrial animals (particularly birds and insects) has been well studied, and consequently their iridescent mechanism and function is well understood (Fox & Vevers 1960, Fox 1976). However, only a few studies have been conducted on the iridescence of marine animals, such as the tapetum lucidum of shark's eyes, used for enhancing vision (Springer & Gold 1989), and the multilayered reflectors in the integument of male sapphirinid copepods, used for attracting conspecific females (Chae & Nishida 1993, 1995). The discovery of iridescence in myodocopin ostracods,

resulting from external diffraction gratings, and its function during courtship (Chapter 6), stimulated further investigations into whether iridescence is more widespread within the Crustacea in general.

Light of different wavelengths is absorbed differently in the marine environment; red light is first to be absorbed, and only blue light penetrates beyond about 200m depth (Denton 1990). For this reason, the species of sapphirinids which reflect many colours inhabit waters nearer the surface than the species which reflect mainly blue light (Chae & Nishida 1995). Because iridescence is reliant upon ambient light, it is not surprising that, within the Crustacea, bioluminescence has attracted more attention than iridescence. Crustacean bioluminescence has long been studied in functional (Herring 1990), chemical (McCapra 1990) and/or evolutionary (Cohen & Morin 1993) terms. Functions for crustacean bioluminescence include predator evasion, for example copepods in response to euphausiids (David & Conover 1961) and intraspecific communication, for example during courtship in ostracods (Morin 1983). Marine bioluminescence is mostly blue or blue-green (Denton 1990).

Another well studied and more common category of light display in crustaceans is pigmentation. Camouflage is a major function of colour pigments in crustaceans, either for concealment from predators, such as isopods from fish (Jormalainen *et al.* 1995), or to mask themselves from prey, such as stomatopods from fish (Steger & Benis-Steger 1988). In fact, many crustaceans living below about 150m are black or red; red coloured animals would appear black in water beyond

the penetration limit for the red component of sunlight. Bright conspicuous pigment colours are also prevalent throughout the Crustacea, such as those used for courtship in fiddler crabs (Hagen 1970), and for warnings to aggressors, such as the meral spots of stomatopods (Reaka & Manning 1987). Bright colour pigments may also be used for camouflage, such as in the red alpheid Synalpheus brucei which harmonizes with its crinoid host (Potts 1915). Some crustaceans are devoid of colour and appear translucent (except for their eyes), such as the ghost crab Ocypode quadrata, which was observed to ingest bioluminescent ostracods and appear bioluminescent itself (Felder 1982), and the amphipod Hyperia galba, which may sometimes become coloured yellow-brown to match its substrate (eg. Schmitt 1965). The colour changes of species of Leander and Palaemonetes (Caridea) are under hormonal control and are affected by the compound eyes and/or possibly the naupliar eye (Knowles 1938).

Crustaceans frequently bear two types of eyes; frontal (naupliar) and lateral (usually compound). The eyes of marine animals may be well adapted to their often relatively dark environment, and may consequently detect extremely low intensity light, beyond the human visual perception range. In fact, some deep-sea animals probably detect daylight down to about 1000m (Denton 1990). Ostracod iridescence appears quite obvious, even to the human eye, under very low light intensities. Therefore, possibilities for functional iridescence within the Crustacea may have a wider scope than previously realised.

After observing many preserved crustaceans from museum

collections, it became obvious that crustacean iridescence is a common phenomenon. This phenomenon may have gone unnoticed in the past because the reflected light is often very precisely directed, as sometimes unusual orientations of the iridescent parts and/or the animals themselves are required. In addition, the high intensity illumination usually used when studying small animals often masks or reduces the effect of the reflected light. Other cases of conspicuous crustacean iridescence may have been neglected because the iridescence was presumed be non-functional, and as such merely an epiphenomenon, such as the transparent fairy shrimps (Anostraca) which conspicuously reflect blue and green light (Schmitt 1973). This study provides a preliminary report on the occurrence and possible functions of iridescence within the Crustacea. The physics behind iridescence is not presented in detail.

2. MATERIALS AND METHODS

(a) Selection of specimens/photography

From preliminary sorting activities of museum marine invertebrate collections, iridescent and non-iridescent species of Crustacea became apparent. Along with iridescent and non-iridescent isopods, which were brought to my attention (S.J. Keable, pers. comm.), species presented in Table 1 were chosen for study. These included a wide range of taxa within the Crustacea.

Specimens were viewed in water under a Zeiss dissecting microscope, using unidirectional white fibre-optic illumination, from all possible angles. Any bright colours displayed must have been the result of physical structures, because the museum specimens were all fixed in 5% formalin and preserved in 70% ethanol, which would have destroyed all colour pigments and bioluminescent compounds. The presence/absence, and description, of iridescence was noted for each species examined (Table 1). Each specimen was extensively studied for iridescence to be assured of an accurate interpretation, because iridescence is precisely directed in some species.

Some iridescent specimens were photographed in water under a Zeiss dissecting microscope and low intensity unidirectional white fibre-optic illumination, using Kodachrome 64 film.

(b) Ultrastructural analysis

Specimens which displayed iridescence from setae/setules, and also specimens bearing non-iridescent setae/setules, were cleaned using five half-second exposures to ultrasound, and dissected. The setae/setules were critical point dried using a Bio Rad CPD 750, coated with gold and examined using a Cambridge Instruments S120 scanning electron microscope (SEM). Specimens which displayed iridescence, and specimens without iridescence, from the exoskeleton of the body and/or appendages were similarly treated and studied under the SEM for surface ornamentation.

Sections of the exoskeleton of <u>Tanais tennicornis</u> and <u>Cohenia</u> sp. 1 (both less than 1cm long) were made by placing specimens on a paper label stuck to a glass slide, then longitudinal sections (about 0.2mm thick) were cut using a razor blade (the blade sank into the paper label, providing a complete cut) (Louis Kornicker, pers. comm.). Other specimens were dry fractured using the technique of Toda *et al.* (1989). Sections were treated as above, and examined under the SEM.

Swimming paddles from specimens of the large portunids <u>Ovalipes molleri</u> (iridescent) and <u>O. australiensis</u> (non-iridescent), and the carapace of <u>Cohenia</u> sp. 1, were fixed overnight in 2.5% glutaldehyde with 2% paraformaldehyde in 0.1M sodium cacodylate buffer, then washed in 0.1M sodium cacodylate buffer. The specimens were subsequently postfixed in 1% osmium tetroxide then dehydrated through an alcohol series and finally 100% acetone. The specimens were embedded in spurr resin and 60nm sections were cut. The sections were stained using lead citrate and uranyl acetate, then examined in a Philips CM10 transmission electron microscope (TEM).

(c) Functional investigations

Three taxa, where iridescence was present in certain species or genera, but not in other closely related species or genera, were examined further to determine whether their iridescence could be functional. These taxa were the isopods and caligoid copepods shown in Table 1, and the portunids in the genus <u>Ovalipes</u>.

The cirolanid isopods were examined under a dissecting

microscope for the position of the iridescence, and its relationship with the compound eyes. In particular, the number and arrangement of ommatidia, and size and shape of the compound eyes of each iridescent and non-iridescent species were documented.

The caligoid copepods are parasites of large sharks and marlin. Sharks and marlin caught at the extensive Port Stephens (New South Wales, Australia) game fishing tournament (25-26 February 1995) were examined for copepod parasites. The species and weight of the host fish, and the total number and distribution of each copepod species over the surface of each fish were recorded. Specimens of each copepod species were examined in water under a dissecting microscope, and the presence/absence, colour and general intensity of the iridescence recorded. The relationship between the presence (and type) of iridescence and the distribution of the copepods on their fish hosts was examined.

From the literature and an examination of specimens held at the Australian Museum, the relationship between iridescence, habitat depth, biogeography and colour pigmentation (of living animals) was examined for species of the portunid genus Ovalipes.

In all of the above functional investigations, male and female specimens of each taxon were studied for sexual dimorphism of the iridescence.

3. RESULTS

(a) Diversity of crustacean iridescence

The colour and position of iridescence, if present, on all specimens studied is recorded in Table 1. Examples of iridescence are shown in Figure 1. Most taxa examined exhibited some type of iridescence, although the spectral reflectance from the cuticular surfaces often appeared fainter, although still prominent, than the more striking setal/setule iridescence. The latter tended to be most intense in the violet-green region of the visible spectrum, and the direction in which light was reflected was precise, often making it difficult to locate. Most cuticular surfaces exhibiting iridescence reflected all colours, although some colours often appeared more obvious than others (Table 1). The colour observed was dependent upon the point of observation, direction of incident light, and orientation of the animal itself. The amphipods <u>Stephonyx pirloti</u> and <u>Waldeckia australiensis</u> reflect white light with a metallic (silver) appearance. All iridescence appeared most obvious when the incident light was very low, and when the iridescent parts were positioned against a black background. In such low light intensities, the colour pigmentation of the living specimens of caligoid copepods examined (see below) was not visible (compare Figure 1 *b* and *c*).

Many of the taxa examined exhibited iridescence which was clearly visible externally. However, some taxa only displayed iridescence from internal, or externally masked, body surfaces

or setules, which could not be seen until the animals were dissected; ie. this iridescence was not visible externally. These latter cases include the iridescence of the externally concealed carapace surface of <u>Nebalia</u> sp., the internal exoskeletal surface of a xanthid cheliped (compare Figure 1 *o* and *p*), and the fourth limb combs of the cylindroleberidid ostracods <u>Tetraleberis brevis</u> (Figure 1 *j*) and <u>Archasterope</u> sp. (hidden within the carapace, although possibly visible ventrally when the carapace is opened). The ostracod halophores (Chapter 5) were the only cases of sexual dimorphism of the iridescence examined, where males exhibited the more striking iridescence.

Of all the taxa examined, only the non-iridescent ostracods <u>Conchoecia belgicae</u>, <u>Loxoconcha australis</u> and <u>Neonesidea globulus</u> do not bear compound eyes.

(b) Mechanisms of crustacean iridescence

Two different mechanisms for producing iridescence within the Crustacea were identified: diffraction gratings on the surface of setae/setules and carapace selvage, and as a result of closely spaced setules (Figure 2 *a*), and multilayer reflectors (Figure 2 *b*).

(i) Diffraction gratings

Diffraction gratings occur on the surface of setae or setules of species from the following taxa in Table 1 which displayed iridescence: Myodocopida (on halophores), Caligoida

(on setae fringing the carapace; Figure 3 *a*, *b* and *c*),
Callianassa (on terminal setae of the small chelipeds,
maxillipeds 2, and periopods 3 and 4; Figure 1 *h*), Isopoda (on
aesthetascs; Figures 1 *g*, and 3 *d* and *e*), and Leptostraca (on
setae fringing the antennal scales). In addition to its
halophores, Euphilomedes carcharodonta bears a diffraction
grating on its selvage, in particular around the rostrum
(Figure 3 *f*) where the selvage is very pronounced in this
species. Other myodocopins may possess similar gratings,
although the selvage is usually too narrow to provide a
prominent iridescent effect. The selvage is a thin extension
of the myodocopin carapace, with a corrugated morphology; in
E. carcharodonta the ridges are about 300nm apart. Non-
iridescent setae/setules of the first and second antennae of
the podocopid ostracod L. australis do not bear diffraction
gratings.

The diffraction gratings on setae/setules consist of a
series of very closely and evenly spaced grooves and ridges,
which split the incident white light into its component
colours (Figure 2 *a*). The grooves, or ridges, are always
spaced less than 700nm apart; ie. their spacings are within
the range of wavelengths for visible light, or less.
Iridescence appeared most intense in cases where the spacings
are less than 400nm (the lowest wavelength for visible light),
such as in the isopod and caligoid examples. In the isopods
examined, the grooves/ridges forming the diffraction gratings
run longitudinally along the setal surfaces; in all other
cases the grooves/ridges run transversely.

The lysianassoid amphipods Stephonyx pirloti and

<u>Waldeckia australiensis</u> reflect silver/white light, with tinges of colours, from the numerous setae of their second antennae, and the tips of their pleopodal (and to some extent uropodal) setae (not clearly visible with high intensity incident light). These setae bear approximately equally spaced grooves (about 80nm apart) in their flattened surfaces which run in two directions, subtending an angle of 60°; both 30° from the exact transverse (perpendicular to the setal length) position (forming bimodal gratings) (Figure 4). Here, the incident light is sufficiently scattered to cumulatively reflect all spectral colours in all directions, thus re-forming white light. However, colours are occasionally observed where scattered light of precise wavelengths do not combine.

The cylindroleberidid ostracod <u>Tetraleberis brevis</u> bears diffraction gratings on its halophores (groove spacings of 125nm), but also produces iridescence from the comb of its fourth limb (Figure 5). This iridescence is the result of the diffraction grating arising from the closely spaced setules, approximately 180nm apart, which occur on the more widely spaced setae).

(ii) Multilayer reflectors

The iridescence which occurs from the surfaces of the body and appendages of <u>Tanais tennicornis</u> (Figure 1 *n*) and <u>O. molleri</u> (Figure 1 *m*), and from the carapace surface of <u>Cohenia</u> sp. is the result of multilayer reflectors. These are structures within the integument which consist of many

alternating light and dark layers as seen in the TEM (Figure 6
b, c). All light layers are of equal thickness, and all dark
layers are of equal thickness (each layer in the order of
100nm in O. molleri), although these two thicknesses are
slightly different. Ovalipes australiensis (non-iridescent)
exhibits an integument without the multilayer structure
(Figure 6 a).

(c) Functional analyses

(i) Cirolanid isopods

The aesthetascs, on the antennules (first antennae), are
always positioned very close to, and overlie, the compound
eyes of the iridescent isopod taxa in Table 1 (Figure 1 f).
The antennules of these taxa are long and thin, reaching over
the complete length of the eyes (Figure 1 f). The non-
iridescent isopods Natatolana woodjonesi and N. corpulenta
exhibit short and broad antennules. There is also a difference
between the compound eyes of the iridescent and non-iridescent
isopods (Figure 7). The eyes of iridescent species are longer
with more ommatidia, arranged in rows, than the non-iridescent
isopods examined. An exception amongst the iridescent isopods
is Plakolana sp.; its iridescence is maroon rather than
violet/blue, and its compound eyes are pink when preserved
rather than brown or black.

(ii) Caligoid copepods

The fishes on which the caligoid copepods were found were all caught near the ocean surface off the New South Wales, Australia, coast, usually in seas of about 1000m depth. The caligoids which exhibit iridescence all aggregate on the surface of their host fishes (Table 2 and Figure 1 *a* and *d*); while those that do not iridesce are distributed randomly over the surface of their host fishes (Table 2). The surface of the host fish where the copepods aggregate appeared tender and discoloured (pink) (Figure 1 *a* and *d*), although to some (lesser) extent the surface of the host is also softened by individually positioned copepods. The caligoids (<u>Pandarus</u> sp.) which do not iridesce lack the setae fringing the carapace which bear the diffraction gratings in the iridescent caligoids.

The most intense caligoid iridescence, of which blue is the most obvious colour reflected, occurs from <u>Gloiopotes longicaudatus</u> (Figure 1 *c*). This copepod bears the most precise caligoid diffraction gratings; the grooves/ridges are very evenly spaced and regular throughout their lengths (compare Figures 3 *b* and *c*). <u>Gloiopotes longicaudatus</u> also exhibits the most colourful pigmentation (purple and red), as opposed to the browns of the other caligoids examined (Table 1).

<u>Gloiopotes longicaudatus</u> was the only copepod to parasitize the external surface of marlin, and often did so in large numbers. The sharks examined sometimes possessed more than one species of parasitic caligoid, although the total

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numbers of caligoid parasites on the sharks never reached as
high as that of some marlin specimens (Table 2).

(iii) Portunid decapods

The portunids in the genus <u>Ovalipes</u> are divided into two
groups (with two sub-groups within each group: A1 and A2, and
B1 and B2) based on morphological similarities (Stephenson &
Rees 1968; Table 3). Group A is exclusively non-iridescent,
group B is exclusively iridescent. However, members of the
sub-group B1 are iridescent over most of their carapace and
appendages, but members of the sub-group B2 show a much
reduced distribution of iridescence (on the frontal teeth and
anterior borders of each carpus, and the general upper
surfaces of each manus and dactylus). The iridescence from the
portunids in group B is very obvious (Figure 1 *m*), and all
colours can be viewed, in both water and air, as the
orientation of the portunid varies. Blue appears particularly
intense, and is only observed when the angle subtended at the
portunid's surface between the incident light source and the
point of observation is large (approaching 180°). The reason
for this angle will be explained later in this paper.

In the above isopods, copepods and portunids, no obvious
sexual dimorphism of the iridescence or compound eyes was
observed.

4. DISCUSSION

(a) Diversity of crustacean iridescence

Iridescence, produced as a result of diffraction gratings or multilayer reflectors, is demonstrated herein to be a more common phenomenon within the Crustacea than previously recognised. Apart from the isopods, diffraction gratings occur on setae/setules which are themselves only visible from certain angles. Multilayer reflectors produce iridescence from parts of the animal which are usually visible from many angles (except in <u>Ovalipes ocellatus</u> and <u>O. guadulpensis</u>). Therefore, diffraction gratings may generally be more practical when the resulting iridescence is only required to be functional by the host animal at certain times, such as when it has a courtship function. In addition to the positional factor, the iridescent light may only be reflected at specific angles.

The hard and rigid nature of the crustacean exoskeleton provides an ideal foundation for physical structures capable of interfering with light waves. Therefore, crustacean iridescence is potentially a wide-ranging occurrence. However, many more taxa should now be studied to provide a more comprehensive account of this subject. Such a study is practical because, unlike bioluminescence and pigmentation, iridescent structures, and consequently iridescence, are retained in the preserved specimens of collections.

The current investigation only examines marine crustaceans, but the potential for fresh and brackish water crustaceans to employ functional iridescence also exists.

The reflectance of ultraviolet light by crustaceans should also be investigated, particularly taxa which efficiently display violet iridescence such as the cirolanid isopods. Additionally, infrared reflection by crustaceans should requires study.

The physical structures which reflect sunlight to produce iridescence may similarly reflect bioluminescent light. This may be especially significant in dark environments such as in deep water or water near the surface outside daylight hours. Crustaceans bearing structures which reflect white light (eg. the amphipods S. pirloti and W. australiensis) may, in particular, reflect bioluminescence, because this would provide a display of the more efficient blue light which would also prevail below about 200m depth in marine environments. Bioluminescence has mistakenly been reported from the antennae (second antennae) of certain amphipods, when in fact the highly reflective surfaces of these antennae are merely reflecting the bioluminescence produced from another region of the amphipod (Herring 1981). Therefore, the reflectance of bioluminescence can be highly effective.

(b) Functions of crustacean iridescence

(i) Cirolanid isopods

The cirolanid isopods examined exhibit a relationship between iridescence and morphology of the antennules, and possibly, to some extent, compound eye morphology. The iridescent isopods bear slender antennules which position

their iridescent aesthetascs over the generally elongated, dark coloured (in incident light), compound eyes, which provide a dark background for maximum contrast of iridescence (Figure 1 *f*). However, a more detailed study of this subject is required. Cirolanid iridescence could have a function in conspecific recognition such as during courtship, stimulating the onset of copulation similar to the iridescence of certain cypridinid ostracods (Chapter 6), or it may serve as a warning (aposematic) colour.

Maybe the iridescence of <u>Plakolana</u> sp. represents an intermediate stage in the evolution, or loss, of cirolanid iridescence. However, the maroon iridescence of this species might elude predators of cirolanids more attuned to the usual violet/blue light.

The cirolanid isopod <u>Cirolana borealis</u> is maximally sensitive to light of wavelength around 495-528 nm (Lindström & Nilsson 1983), ie. blue, and could therefore detect cirolanid iridescence, although this is probably an adaptation to its deep-sea environment.

(ii) Caligoid copepods

The iridescence of the caligoid copepods examined appears to be acting as an aggregation mechanism (social aggregation; Ritz 1994); only the iridescent caligoids examined were observed to aggregate. This iridescence is positioned on the lateral edges of the copepods and so would be easily visible to conspecifics, which bear dorsolateral compound eyes. Caligoid aggregation results in an increased softening of the

host's surface, which increases the copepod's feeding efficiency. This occurs because the collective digestive fluids of a group of copepods feeding from a small area stimulates an immune response of the host, comparable to mites parasitic on cattle (Stromberg & Fisher 1986).

The reproductive potential of caligoids may also be increased when aggregated. Blue marlin, for example, tend to swim fast (up to 225cm s^{-1}) at depths greater than 50m, and slow (15-25cm s^{-1}) when within 10m of the surface, and a similar situation exists for free-swimming sharks (Block *et al.* 1992). Therefore, when the iridescent caligoids are near the surface of the ocean on their host, the host fish is also swimming at its slowest speed. Hence conditions for caligoid mating are optimal at this depth. It is consequently beneficial for the copepods to be arranged in a group, so that when these optimal mating conditions arise, the copepods may take full advantage and copulate immediately, before conditions change.

The only caligoid found to be parasitic on the marlin Makaira indica, M. mazara and Tetrapturus audax examined in this study, was Gloiopotes longicaudatus. This copepod sometimes occurred in high numbers when compared to the low abundance of the other caligoid parasites. Similar high numbers of G. longicaudatus were found on M. indica from Queensland waters (Speare 1994). Gloiopotes longicaudatus also exhibits the most efficient blue iridescence of all the caligoids examined. Maybe its highly efficient iridescence is responsible for the success of this copepod, acting as a conspecific signal to initiate social aggregation. Purple and

red pigmentation is present in <u>G. longicaudatus</u> (Figure 1 *b*). This may add to the colour display of this copepod, and/or serve to camouflage the copepod against its host, while its iridescence is positioned laterally in full view of conspecifics. However, there is no evidence that the fixed colour pigments of this copepod are cryptic as <u>G. longicaudatus</u> was found attached to dark and light areas of its hosts.

(iii) Portunid decapods

Portunids in the genus <u>Ovalipes</u> show a relationship between depth and iridescence. Species which inhabit deep water (group B1; Table 3) exhibit maximum iridescence. However, two closely related species from shallow water (group B2; Table 3), also exhibit iridescence, although only from restricted regions of their bodies and appendages. There is no relationship between iridescence and biogeography in this genus (ie. iridescence is not restricted to specific geographical localities).

The most likely function for the iridescence of species of <u>Ovalipes</u> is conspecific signalling, to simply attract the attention of a potential mate in a dimly lit environment (see "Distribution of light in the sea" section).

The species of <u>Ovalipes</u> exhibiting maximum iridescence exclusively inhabit deeper waters (between about 80 and 475m) because in this zone the advantages of iridescence which cannot be easily concealed outweigh the disadvantages. Between about 80 and 475m, the ambient light is so reduced in its

intensity, and filtered, that potential predators of Ovalipes probably bear subordinate eyes, and increasingly rely on senses other than vision to detect their prey. However, iridescence still occurs in such low light regimes. Colour pigmentation may not be practical in attracting conspecifics (a more startling effect is required), but may be more useful to enhance concealment from other animals, at depths between about 80 and 475m.

The species of Ovalipes displaying restricted iridescence (group B2) probably do not display the maximum iridescence due to the presence of predators with efficient visual systems in their "light" environments. Therefore, species in the group B2 bear multilayer reflectors in areas of the cuticle which may be concealed at times when iridescence is not required.

The above three taxa (cirolanids, caligoids, and Ovalipes) should be fully examined and their light adaptation characters added to a phylogenetic analysis produced from many morphological and/or genetic characters for each group (similar to the case of the cypridinid ostracods; Chapter 6). The direction of evolution of the light adaptation characters may then be revealed.

The potentially high occurrence of crustacean iridescence is important because of its functional possibilities. The fact that much of the crustacean iridescence reported herein is blue (or optimally blue), increases its potential of being functional. This is substantiated by the fact that marine bioluminescence is mainly blue or blue-green. Blue light

transmits maximally through sea water, and below about 200m daylight (ie. the incident light source) is exclusively blue (Denton 1990). Electroretinogram recording techniques (Donner 1971) could be carried out on the eyes of iridescent crustaceans optimally reflecting blue light, to test whether they also optimally perceive blue light of the same wavelength. If an animal optimally reflects and perceives light of the same wavelength, then this would provide evidence towards the case for functional iridescence. However, determining the actual function of the iridescence is more complicated, partly due to the difficulty in reconstructing natural conditions in the laboratory. Also, for iridescence to be functional, the animals in question must be active during daylight hours (or at least dawn and/or dusk), unless bioluminescence can provide an incident light source or animals positioned near to the surface can utilize moonlight.

While a role in mating is one function of crustacean iridescence (Chapter 6), other functional possibilities exist.

Although flashes of light can confuse a predator, it is doubtful that marine iridescence could startle a predator in the capacity of which bioluminescence is capable. Iridescence may be useful in attracting prey or cleaning commensals, provided the properties of the light which is displayed maximally is optimally detected by such beneficials.

Another possible function for crustacean iridescence is in a territorial role. Colour pigments are well-known to provide warnings to potential invaders of an animal's territory in many situations, and it is conceivable that iridescence may have comparable effects on invading

conspecifics. In a similarly aggressive manner, iridescence could be used to warn off competitors for a mate. However, in these cases, the iridescence would probably require concealment, only to be displayed when required, similar to the flash colouration of certain frogs and stick-insects (Cott 1966). Also, in the last potential function, along with functions during mating, sexually dimorphic iridescence would be expected such as is evident in certain cypridinid ostracods (Chapter 6). However, iridescence may not necessarily require concealment to avoid an increased predation rate. Blackbirds with a bright red wing patch are favoured over those without the red patch by a decreased risk of predation (Götmark 1994). Additionally, there may be multiple functions for the iridescence of a particular species, and iridescence may be employed in conjunction with other mechanisms to achieve a function (eg. with pheromones to attract a mate).

Borderline cases, where iridescence is only marginally visible, provide an obstacle for automatically assuming iridescence to be functional whenever it can be viewed externally. These cases include the carapaces of certain myodocopid ostracods (Table 1), which exhibit iridescence which appears faint when compared to the prominent iridescence of <u>Sapphirina</u> sp., for example. However, iridescence which appears faint to human eyes, may appear quite different to individuals of the species exhibiting the iridescence against its natural background. Indeed, conspicuous iridescence may not, in some circumstances, be suitable. It may be that there is selection for the minimum level of iridescence required for its function. Under certain circumstances, higher levels of

iridescence could be maladaptive in attraction of predators. Additionally, iridescence that exhibits a relatively low intensity may consequently have a wide angular field. This may be most suitable in some circumstances, such as when the location of the intended recipient animal is unknown. Also, it could be argued that if iridescence is an epiphenomenon, ie. the structure causing iridescence has some other function, then the advantages of that other function may outweigh the disadvantages of the accidental iridescence. However, the structure would most probably be altered slightly, following the actions of selective pressures, to prevent externally visible iridescence, but allow the intended function to continue.

In conclusion, I hypothesize that when an animal inhabits an environment where light is present and can be detected either by conspecifics or other organisms living in that environment, and the animal exhibits iridescence externally, then that iridescence is probably functional. If this is not the case, and the iridescence has no use to the host animal, then surely unnecessarily attracting attention to itself would be disadvantageous, and the iridescence would be lost following the actions of selective pressures. This hypothesis is exemplified by the case of the xanthid decapod. The species of xanthid examined exhibits a layered construction to its cuticle, which by its very nature (a multilayer reflector) causes iridescence, ie. it is an accidental multilayer reflector (the iridescence is an epiphenomenon). This iridescence is highly visible on the internal exoskeletal surface (Figure 1 *p*), but is biologically insignificant as no

potential enemies may view this iridescence. However, the expected iridescence from the xanthid's external exoskeletal surface is prevented (Figure 1 o) by an opaque layer within the cuticle, ie. with a different chemical composition to the corresponding cuticular layer of O. molleri, for example, which transmits the incident light, permitting iridescence. The xanthid, therefore, remains camouflaged in its environment (enhanced by the presence of colour pigments which match the xanthid's background). This is analogous to the precluded external iridescence of many mollusc shells. The "internal" (or externally concealed) iridescence produced from the comb of the cylindroleberidid ostracods, and the internal surface of the nebaliacean carapace (Table 1) under incident light, provide a similar allegory.

(c) Distribution of light in the sea

At any point in the water column from where light is radiated, the light which is travels vertically downwards reaches the furthest (Denton 1970; Young 1983), in the absence of any bioluminescent light (Figure 8). Light reflected from an iridescent source would therefore travel furthest if reflected in a vertically downward direction. Consequently, it is beneficial to the iridescent animal to reflect the light which is potentially displayed with maximum intensity, ie. the light of wavelength intended to be optimally functional (usually blue), vertically downwards in the water column. Therefore, selective pressures may determine the position and orientation of the structures causing iridescence, and a

modification of behaviour, so that such a result is achieved. This explains the distribution of iridescence of O. molleri. The upper surfaces of this portunid, which swim with an orientation at an angle to the horizontal, reflect blue light at the greatest angle of reflection. Therefore, blue light, the only colour which is present at the depths where these portunids live, is reflected almost vertically downwards in the water column, ie. the direction permitting the furthest distance of travel of light. Hence, a swimming individual of O. molleri (close to the sea floor) would attract a conspecific on the sea floor.

If bioluminescent light is reflected then the animal should be similarly orientated to reflect the light vertically downwards, and positioned directly below the iridescent source to obtain the highest intensity incident light.

However, this maximum reflectance situation may not always be practical; an iridescent animal may not always know the position of the recipient animal prior to signalling. An advantage, however, of reflecting light maximally towards the surface (ie. when the recipient animal is above the animal displaying the iridescence in the water column) is that the background (ie. the deep sea) is darker than the ocean surface, and therefore the contrast of the iridescence against its environment is greater.

This distribution of light in the sea (Figure 8) is highly uniform, in both colour and angular distribution (only light intensity varies) in deep water. However, in near-surface waters, the light field is highly complex, dependent upon solar elevation, cloud cover, and sea state (eg.

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phytoplankton density), and may consequently vary (Young 1983). Therefore, in shallow water iridescence is dependent upon the variable conditions, and so eyes should be more accommodating, but in deeper waters, iridescence and eyes may be much more finely tuned and consequently provide a more efficient light display/detection system. However, this advantage for iridescence in deep water may be outweighed by the sometimes disadvantageous low ambient light intensity.

(d) Advantages of iridescence as a functional tool in marine environments

The three main mechanisms for light display in the marine environment are pigmentation, bioluminescence, and iridescence. In terms of the intensity of the resulting light displayed, bioluminescence is the most intense, iridescence intermediate, and pigmentation the least intense. Unlike bioluminescence, and to some extent chromatophores, the employment of iridescence expends no or little (eg. to orientate the iridescent structures) energy.

Pigmentation in marine animals may be most useful in water near the surface where there is a relatively high ambient light intensity (which is required for pigments to be conspicuous), in mid-deep water (eg. 50-200m) only to provide camouflage against their background, or in deep water (below 200m), where red light does not penetrate, and where red or black pigmentation makes them appear invisible. Bioluminescence, although capable of displaying the most intense light, is ineffective in relatively high light

regimes, and is therefore exclusively employed in deep water or water near the surface at night (or in times of low ambient light intensities, such as dawn or dusk). Iridescence, however, may be the most efficient means of displaying light or colour in marine environments where the ambient light is reduced, but not absent (where pigmentation becomes invisible and the effect of bioluminescence is sub-optimal). In some dimly lit environments, iridescence may be employed rather than bioluminescence, because bioluminescence may be so outstanding as to attract unwanted attention in addition to that of the intended. Similarly, in other situations (high light regimes), pigmentation may be selected for over iridescence.

Iridescence plays a role in, or is linked to, the reproductive process of certain crustaceans. This function is most important in the survival of a species, as epitomized by the intricate courtship behaviour of many animals. However, iridescence which is used to attract a conspecific may also attract an enemy. Similarly, if iridescence is used as a warning to a targeted individual, it may attract the unwanted attention of other individuals. Therefore, whenever iridescence is employed in nature, it must carry with it disadvantages which are outweighed by its advantages. Hence, in relatively well lit marine environments, where co-inhabitants often possess good visual perception, the iridescence of a crustacean may be hidden from external view (eg. tanaids may hide in tubes). Consequently, the employment of iridescence must lead to compromises in an animal's morphology and behaviour, and its depth, geographic and

microhabitat distribution. Ultimately, iridescence is a tool which may be utilized in an animal's environment where light is a major stimulus.

Acknowledgements

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Figure 1. Examples of iridescent crustaceans. (*a*) Caligoid copepods, <u>Gloiopotes longicaudatus</u>, parasitic upon the posteroventral surface of a striped marlin, <u>Tetrapturus audax</u> (tail to the left of picture) (photograph by R.Blayden). (*b*) Adult <u>Gloiopotes longicaudatus</u>, living specimen, on <u>T. audax</u> under high intensity white light. (*c*) Close up of area arrowed in (*b*) under very low intensity white light; blue iridescence is displayed from setae fringing the carapace (colour pigments are no longer visible). (*d*) Iridescent caligoid copepods, <u>Dinemoura latifolia</u>, parasitic upon a mako shark, <u>Isurus oxyrinchus</u> (tail to the right of picture) (photograph by R.Blayden). (*e*) Close up of a section from (*d*) (photograph by R.Blayden). (*f*) <u>Bathynomus</u> sp. from 1000m depth, living specimen, anteroventral view of head, left antennule arrowed (photograph by R.Steene). (*g*) Lateral view of the ventral surface (mid way along the length) of a antennule from <u>Bathynomus</u> sp.; aesthetascs are exhibiting blue iridescence. (*h*) Ventrolateral view of the terminal setae of pereiopod 3 from <u>Callianassa arenosa</u>, displaying mainly red iridescence (such a view may be obtained from the entrance of this callianassid's burrow). (*i*) Distal setae (d-, e- and s-) of a first antenna of an adult male <u>Azygocypridina lowryi</u> under very low intensity white light; iridescence is displayed from halophores (setules). (*j*) Comb of a fourth limb from <u>Tetraleberis brevis</u>, in motion, under low intensity white light. (*k*) <u>Sapphirina</u> sp., dorsal view, under low intensity white light. (*l*) <u>Ovalipes molleri</u>, died just prior to this picture being taken, dorsal view, under high intensity white light; colour pigmentation is highly visible (photograph by

H.McLennan, Australian Museum). (*m*) Swimming paddle of <u>O.</u> <u>molleri</u>, close-up of region arrowed in (*l*), of a specimen preserved in 70% ethanol for many years, under low intensity white light; all colour exhibited is the result of iridescence; both the incident light and reflected light photographed are close to the perpendicular or reflector normal (blue light is not observed at these angles). (*n*) Anterior region of <u>Tanais tennicornis</u>, preserved in 70% ethanol, under low intensity white light. (*o*) View of the external surface of a xanthid cheliped. (*p*) View of the internal surface of the same section of a xanthid cheliped as shown in (*o*). Scales: (*a*) = 100mm; (*b*) = 5mm; (*c*), (*g*) and (*k*) = 1mm; (*d*) = 200mm; (*e*) and (*l*) = 50mm; (*f*) = 20mm; (*h*) - (*j*) and (*m*) - (*p*) = 0.5mm.

Figure 2. Physical structures causing iridescence in crustaceans. (*a*) Diffraction grating, separating the incident white light into different spectral orders (after Hutley 1982). The colour and spectral order observed depends upon the point of observation; eg. the longer wavelength red light can be seen at point *y*, and the shorter wavelength blue at point *x* within the first order spectrum. Resolution is increased with an increasing number of grooves to the inch (Hutley 1982). (*b*) Section through a multilayer reflector (after Land 1972); examples of red and blue wave profiles are illustrated. Shaded layers (*d*) are all the same thickness (d_h) and represent a high refractive index material (n_h); unshaded layers (*l*) are also all the same thickness (d_l) and represent a lower refractive index material (n_l). In the quarter-wavelength stack, light reflected from each interface interferes constructively when $n_h d_h = n_l d_l = \lambda/4$ (Land 1972). In this example, the longer wavelength red interferes constructively at the angle of incidence illustrated.
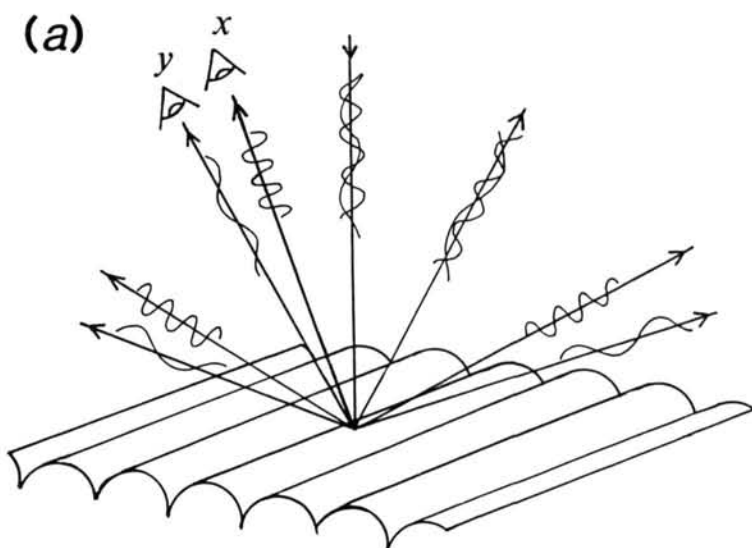
(a)

x

y

(b)

h

l

h

d_h

l

h

l

d_l

h
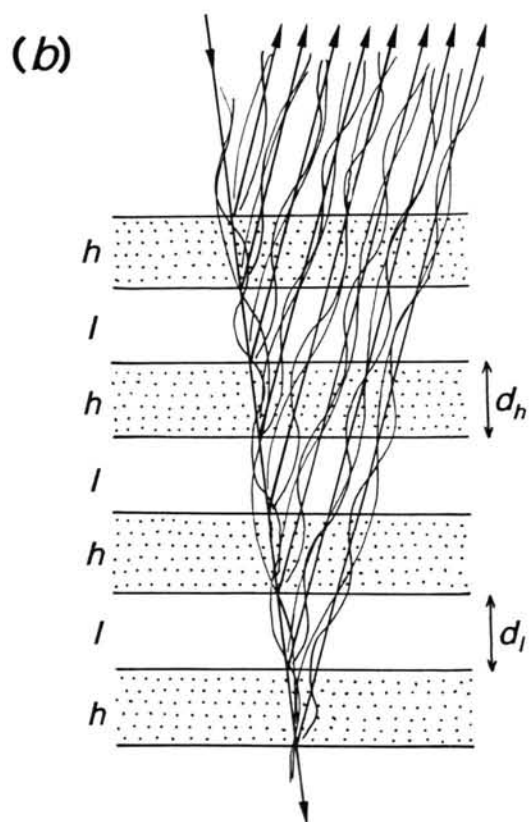
Figure 3. Scanning electron micrographs of examples of crustacean diffraction gratings. (*a*) <u>Gloiopotes longicaudatus</u>, ventral view, showing position of setae fringing the carapace (example arrowed). (*b*) Surface of a seta of <u>G. longicaudatus</u> from position arrowed in (*a*). (*c*) Surface of a seta of <u>Dinemoura latifolia</u> from a similar position as seta shown in (*b*); grating is less efficient as such. (*d*) Section near base of antennule of <u>Bathynomus immanis</u>, ventral view (base is to the left of the picture); aesthetascs (an example is arrowed), lying in a channel, are thin walled and flattened. (*e*) Surface of an aesthetasc from (*d*); many grooves which comprise the diffraction grating are shown (not the larger vein-like structures), which run longitudinally. (*f*) Section of selvage on the rostrum of <u>Euphilomedes carcharodonta</u>. Scales: (*a*) = 2mm; (*b*) and (*f*) = 10μm; (*c*) = 20μm; (*d*) = 200μm; (*e*) = 5μm.
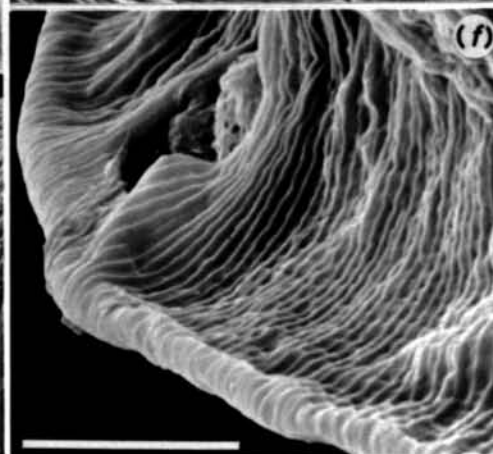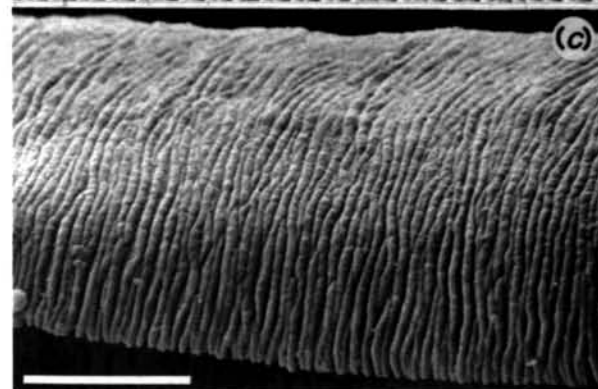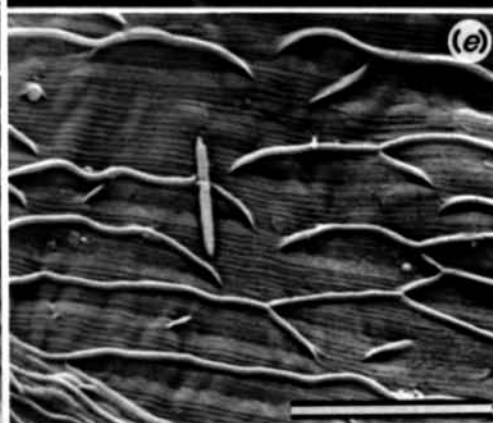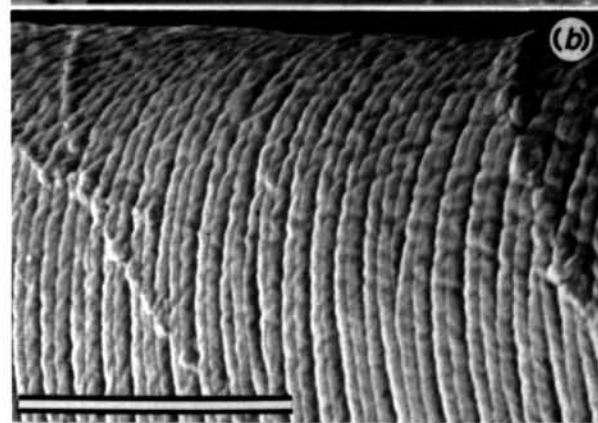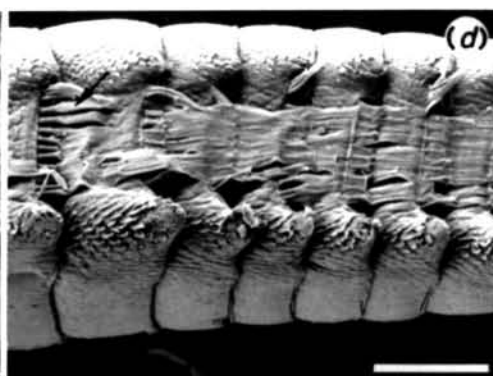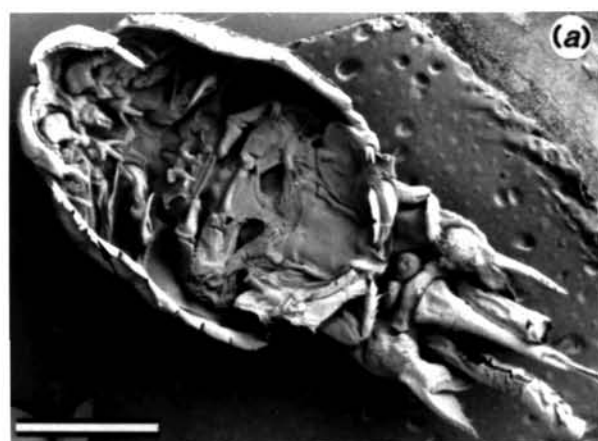
Figure 4. Scanning electron micrograph of setae from the second antenna of <u>Waldeckia australiensis</u> (Amphipoda). Grooves run in two directions on the flattened setal surfaces, forming a bimodal grating. Scale = 2μm.

Figure 5. Scanning electron micrograph of the comb of a fourth limb from the cylindroleberidid ostracod _Tetraleberis brevis_; the setules (running vertically) arising from the setae (running horizontally) act as the ridges of a diffraction grating. Scale = 20μm.
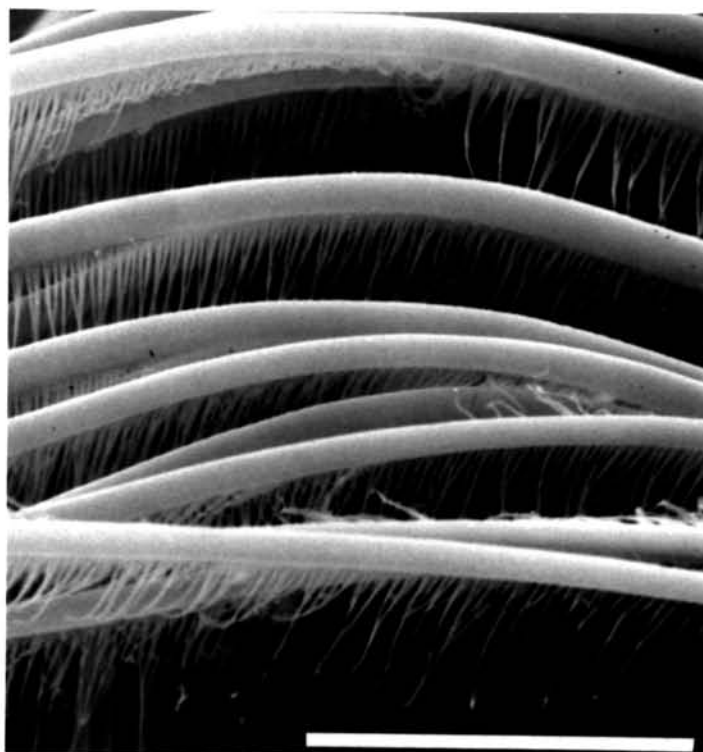
Figure 6. Transmission electron micrographs of the integument of the swimming paddles of adult portunid decapods; c represents epicuticle. (*a*) <u>Ovalipes australiensis</u> (non-iridescent); multilayer reflector is absent. (*b*) <u>Ovalipes molleri</u> (iridescent), bearing a multilayer reflector (area r) in the position arrowed in (*a*); m represents examples of pore canals. (*c*) Close up of a section of the multilayer reflector of <u>O. molleri</u>; from area r in (*b*). Scales: (*a*) and (*b*) = 5μm; (*c*) = 500nm.

Figure 6. Transmission electron micrographs of the integument
of the swimming paddles of adult portunid decapods; c
represents epicuticle. (a) Ovalipes australiensis (non-
iridescent); multilayer reflector is absent. (b) Ovalipes
molleri (iridescent), bearing a multilayer reflector (area r)
in the position arrowed in (a); m represents examples of pore
canals. (c) Close up of a section of the multilayer reflector
of O. molleri; from area r in (b). Scales: (a) and (b) = 5μm;
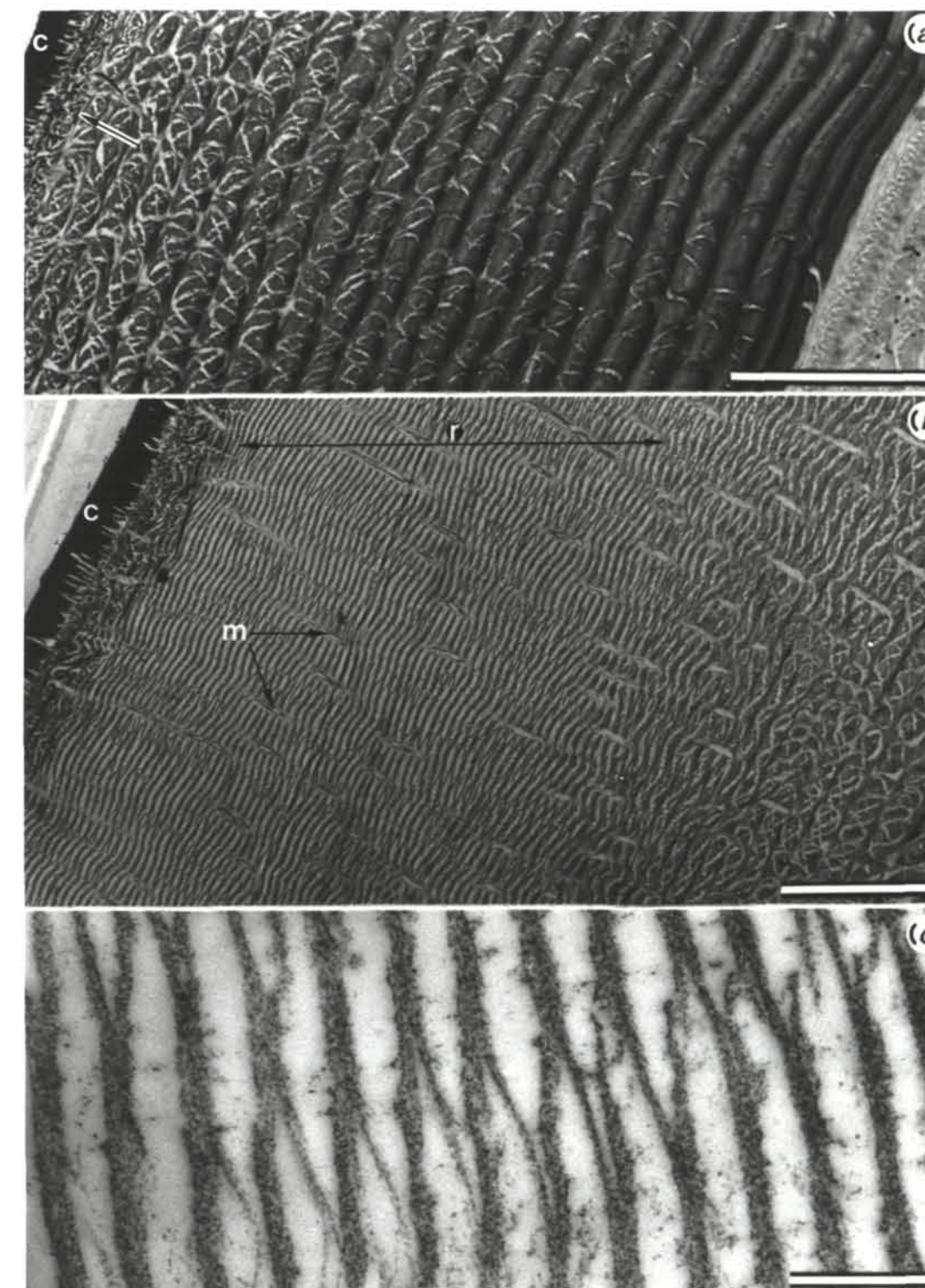(c) = 500nm.

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Figure 7. Right compound eyes of preserved cirolanid isopods, lateral views, anterior to the right. Ommatidia may be hexagonal or square, and all eyes appear dark brown (the cirolanid body is generally pale in colour) or black, except for <u>Plakolana</u> sp., which is pink, and <u>Dolicholana</u> sp., which is pale brown. Scale bar = 0.5mm; R = length of compound eye/total length of isopod.
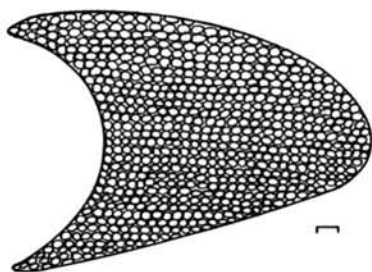
Bathynomus immanis
R = 0.0068
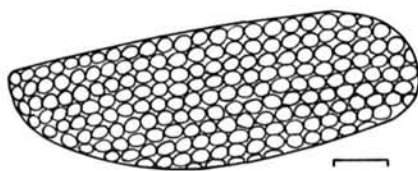
Booralana sp.
R = 0.0088
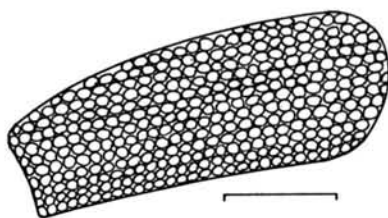
Aatolana schioedtei
R = 0.0073
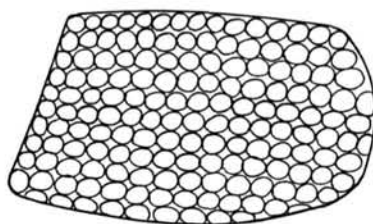
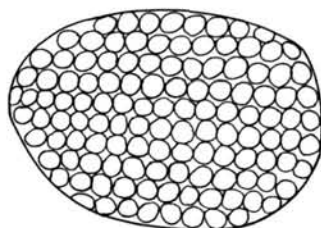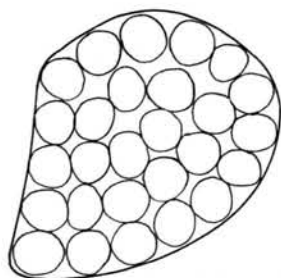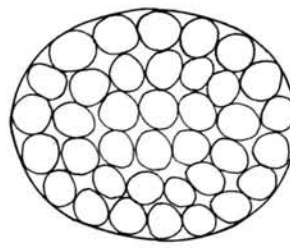Aatolana sp. cf. rapax
R = 0.0059
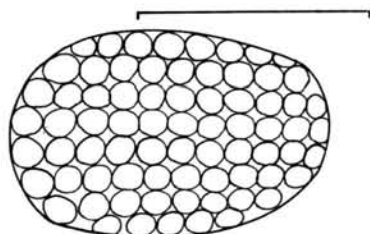
Plakolana sp.   R = 0.0084

Dolicholana sp.
R = 0.0046

Natatolana woodjonesi
R = 0.0046

Natatolana corpulenta
R = 0.0055

Figure 8. Example of a distribution of radiance in the ocean from a single point I (after Denton 1970). The three dimensional distribution is given by the area formed by revolving the dotted area S around the axis UD (Denton 1970).
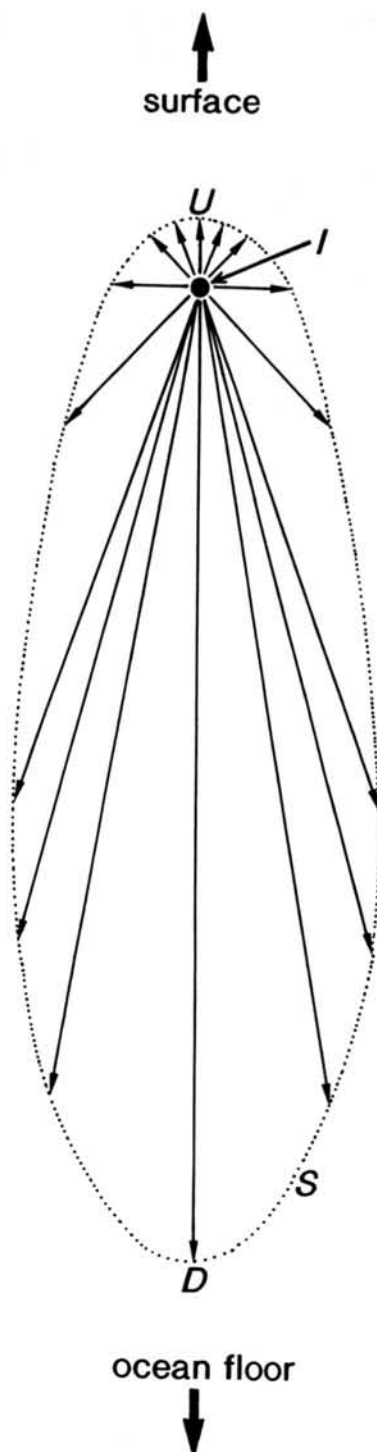
Table 1. Material examined (marine).

Taxa	Depth	Iridescence
Ostracoda		
Podocopida		
Loxoconcha australis	10m	Absent.
Neonesidea globulus	10m	Absent.
Myodocopida		
Conchoecia belgicae	Shallow	Absent.
Azygocypridina lowryi	200m	All colours, from halophores.
Skogsbergia sp.	3m	Blue, from halophores; all colours, from carapace surface.
Cohenia sp.	25m	Blue, from halophores; all colours, from carapace surface.
Cypridina sp.	1m	Blue, from halophores; all colours, from carapace surface.
Euphilomedes carcharodonta	Shallow	Blue/green, from halophores; blue, from selvage.
Euphilomedes sp.	10m	Green, from halophores; blue, from selvage.
Tetraleberis brevis	10m	Violet/green from halophores; all colours (blue brightest), from comb of 4th limb.
Archasterope sp.	10m	Blue/green, from halophores; all colours (blue brightest), from comb of 4th limb; all colours, from carapace surface.
Rutiderma sp.	3m	Blue, from halophores; all colours, from carapace surface.
Sarsiella sp.	10m	Green, from halophores.
Copepoda		
Poecilostomatoida		
Sapphirina sp.	Shallow	All colours (mainly yellow), from body surface.
Caligoida		
Gloiopotes longicaudataus	Mainly shallow	All colours (blue brightest), from setae fringing carapace.
Dinemoura latifolia	Mainly shallow	All colours, from setae fringing carapace.
Dinematura braccata	Mainly shallow	All colours, from setae fringing carapace.
Pandarus sp.	Mainly shallow	Absent.

Malacostraca
  Decapoda
    Ovalipes molleri   154m   All colours, from
                                       exoskeleton of carapace
                                       and appendages.

    Ovalipes australiensis   16m   Absent.
    xanthid sp. (part)   15m   All colours, only
                                       visible internally from
                                       cheliped exoskeleton.

    Chaceon bicolour   1423m   Absent.
    Dardanus arrosora   115m   Absent.
    Callianassa arenosa   4m   All colours, directed
                                       ventrally from terminal
                                       setae of: small
                                       cheliped, maxilliped 2,
                                       and periopods 3 and 4.
  Isopoda
    Natatolana woodjonesi   16m   Absent.
    Natatolana corpulenta   15m   Absent.
    Plakolana sp.   20m   Maroon, from
                                       aesthetascs.

    Dolicholana sp.   Unknown Violet/blue, from
                                       aesthetascs.

    Aatolana sp. cf. rapax   21m   Violet/blue, from
                                       aesthetascs.

    Aatolana schioedtei   10m   Violet/blue, from
                                       aesthetascs.

    Booralana sp.   150m   Violet/blue, from
                                       aesthetascs.

    Bathynomus immanis   200m   Violet/blue, from
                                       aesthetascs.

    Bathynomus sp.   1000m   Violet/blue, from
                                       aesthetascs.
  Amphipoda
    Stephonyx pirloti   100m   (White, from setae of:
                                       2nd antenna, pleopods).

    Waldeckia australiensis   50m   (White, from setae of:
                                       2nd antenna, pleopods).
  Cumacea
    Diastylis helleri   Shallow Absent.
  Tanaidacea
    Tanais tennicornis   10m   All colours, from
                                       exoskeleton of body and
                                       appendages.

    Tanais sp.   20m   All colours, from
                                       exoskeleton of body and
                                       appendages.

    Whiteleggia stephonsoni   Shallow All colours, from
                                       exoskeleton of body and
                                       appendages.
  Leptostraca
    Nebalia sp.   10m   All colours, from setae of
                                       antennal scale and
                                       internal surface of
                                       carapace.

Table 2. Occurrence of caligoid copepods and their
distribution on fishes (sharks and marlins) caught near the
ocean surface off the New South Wales coast, Australia.

G = aggregated (in groups), I = randomly distributed (as
individuals), G.long. = Gloiopotes longicaudatus, D.lati. =
Dinemoura latifolia, D.brac. = Dinematura braccata, Pan.sp.
= Pandarus sp.

Host fish	Number and distribution of copepods			
	G.long.	D.lati.	D.brac.	Pan.sp.
MARLIN:				
Tetrapturus audax, 140kg	432 G	-	-	-
T. audax, 75kg	99 G	-	-	-
T. audax, 50kg	18 G	-	-	-
T. audax, 75kg	26 G	-	-	-
Makaira indica, 96kg	250 G	-	-	-
Makaira mazara, 163kg	6 G	-	-	-
M. mazara, 102kg	17 G	-	-	-
SHARKS:				
Carcharhinus obscurus, 218kg	13 G	-	12 G	10 I
Carcharhinus tilstoni, 144kg	6 G	-	1 I	1 I
Sphyrna zygaena, 76kg	-	3 G	2 G	3 I
Isurus oxyrinchus, 105kg	-	23 G	-	-
I. oxyrinchus, 76kg	-	11 G	-	-
I. oxyrinchus, 96kg	-	21 G	-	-
Prionace glauca, 83kg	-	6 G	7 G	1 I
Galeocerdo cuvieri, 275kg	-	1 I	2 G	22 I

Table 3. Species groups within the genus _Ovalipes_ (Decapoda: Portunidae), based on morphological similarities (Stephenson & Rees 1968), and their geographical locations. Precise depths are quoted when available.

Species	Depth	Distribution
GROUP A1: (non-iridescent)		
O. punctatus	Shallow	Japan and China.
O. trimaculatus	Shallow	Eastern and western South America, eastern and western South Africa, Indian Ocean.
O. catharus	Shallow	New Zealand, southern Australia.
O. australiensis	0-55m	Australia.
O. elongatus	Shallow	Lord Howe Island, Kermadec Islands (South Pacific Ocean).
GROUP A2: (non-iridescent)		
O. georgei	Shallow	Western Australia.
GROUP B1: (iridescent)		
O. iridescens	80-204m	South Africa, Indonesia, Japan, southern Australia.
O. molleri	135-475m	Southeastern Australia.
GROUP B2: (restricted iridescence)		
O. ocellatus	Shallow	Eastern North America.
O. guadulpensis	13-46m	Eastern North America, Gulf of Mexico.

CHAPTER 9

General conclusions

1. Two new cypridinid genera, *Cohenia* and *Lowrya*, containing the species *C. taiti* and *L. kornickeri*, and a new species belonging to the cypridinid genus *Vargula* (*V. karamu*) are established. These are all scavengers from Australian seas.

2. The genus *Cohenia* bears an unusual type of sensillum, here termed the trichocoel. Each consists of a spherical cavity with a very fine, stiff seta, and occur in rows in the carapace. They are probably velocity detectors, which perceive either steady fluid drainage motion or acoustic motion in the surrounding water.

3. The furca is used as a major feeding tool in scavenging cypridinids.

4. The hard section of the dorsal body wall almost adjacent to the furca in all myodocopins is here termed the sclerosome. This probably functions as a shield against the environment when the carapace is opened.

5. The central adductor muscles of myodocopins, and possibly other ostracods, are arranged as a group of small muscles, rather than a single large muscle, to allow areas of the

anterior, ventral, and posterior carapace margins to open differentially to each other. Hence, the body gains maximum protection from the carapace during any activity.

6. The sensory seta of the fifth article of the myodocopin first antenna is termed the s-seta. Setules of the s-seta, and other long first antennal setae, are composed of a series of very fine rings, surrounded by probably an elastic material, and exhibit a terminal pore. Each of these setules is termed a halophore. The halophores of one first antenna are collectively termed the halothalium.

7. The grooved external surface of a halophore acts as a diffraction grating, and causes highly efficient iridescence. This iridescence is functional during courtship in at least some cypridinids.

8. The cypridinids have evolved with light as a major stimulus. It is suggested that iridescence is a precursor to cypridinid bioluminescence, and the myodocopin compound eye may have evolved independently.

9. Some cypridinids, at least, copulate in a position where the ventral margins of the carapace meet, and anterior ends are opposite. The male and female mandibular claws and furcae join, and the latter are forced posteriorly so that genitalia become directly opposite, within reach, and without impediments. Copulation is completed in about five seconds.

10. Iridescence is widespread throughout the Crustacea, occurring as a result of external diffraction gratings, or internal multilayer reflectors. This iridescence is probably functional in cases where the iridescence is visible in the host animal's environment and where other animal species capable of detecting this iridescence cohabit.