JOURNAL OF CRUSTACEAN BIOLOGY, 24(3): 409–421, 2004

LARVAL DEVELOPMENT AND COMPLEMENTAL MALES IN *CHELONIBIA TESTUDINARIA*, A BARNACLE COMMENSAL WITH SEA TURTLES

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A B S T R A C T

That some barnacles are obligate commensals of sea turtles has long been known; however, little attention has focused on understanding the details of this association. In particular, early life-history traits of turtle barnacles, which may be key to establishing the association, have not been well studied. Here we present the first complete description of larval development in a turtle barnacle. Embryos were collected from *Chelonibia testudinaria*, a cosmopolitan and conspicuous species, and reared through metamorphosis in the laboratory. We followed sequential molting and described each stage using light microscopy. Development proceeded over nine days at 25°C and the characteristic cirriped pattern of six naupliar stages followed by a cyprid larva was observed. Implications for the association of barnacles with sea turtles are discussed relative to spatio-temporal aspects of cyprid attachment and the life history of sea turtles. Barnacle reproduction typically involves cross-fertilization between hermaphrodites but small complemental males occur in some species. In *C. testudinaria*, small individuals attached to hermaphrodites were confirmed to be exclusively male. They were located in the external depressions between the shell plates of hermaphrodites, within pits perhaps specialized for their settlement. This attachment location, external to and below the orifice of the hermaphrodite shell, is unique for complemental males. It is not known whether the males of *C. testudinaria* remain small permanently or eventually grow to become hermaphroditic, as do protandric complemental males in *C. patula*. Their small size may provide advantages in reducing drag and their attachment location may afford protection from removal.

Barnacles are atypical among maxillopod crustaceans in that they are hermaphroditic, and they are unusual among sessile animals in being copulatory (Anderson, 1994). Most barnacle species are not self-fertile, because they are either sequential hermaphrodites or they alternate between male and female modalities during breeding (Anderson, 1994). Therefore, barnacles generally cross-fertilize with receptive neighbors and thus need to settle in aggregations, the difficulty of which is compounded by their dispersion in the plankton during larval development. Barnacles that specialize as commensals have the additional complication of needing to locate a suitable host that may be scarce and is often mobile.

Within species, obligately commensal barnacles are narrow in their selection of hosts (Ross and Newman, 1973; Lewis, 1978), but as a group they associate with a surprisingly wide array of invertebrate and vertebrate taxa: sponges, corals, hydrozoans, molluscs, decapod crustaceans, sea urchins, whales, dolphins, manatees, sea snakes, and sea turtles. For epizoic barnacles, the nature of these associations appears to be phoretic (carried or transported by another) as opposed to parasitic in the sense of drawing nutrition from their hosts (Anderson, 1994; for exceptions see Johnstone and Frost, 1927; Moyse, 1971; Ross and Newman, 1995). This is in contrast to the endozoic rhizocephalan cirripeds that feed on their hosts (Høeg, 1995). The commensal habit has almost certainly arisen many times in barnacle evolution, probably as a response to predation (Foster, 1987). However, large gaps in our understanding remain in identifying the proximate determinants of this associative behavior. Early life-history events are certainly critically important, and yet we know virtually nothing about them. In fact, the complete life cycles of epizoic barnacles are known for only a few species (Moyse, 1961; Molenock and Gomez, 1972; Lang, 1979).

Larval development in thoracican cirripeds includes seven instars. The first six are nauplii of which the first one or two may be retained in the mantle cavity of the adult before being released in the plankton to feed and swim. The seventh is a cyprid that does not feed and is specialized for

site selection and attachment. The larval period in barnacles varies by region and species. In coastal temperate regions it is typically 14 to 21 days depending on the species (Bassindale, 1936) and in warm seas 5 to 11 days (Anil *et al*., 2001). Open ocean representatives attach to floating objects and have a larval period that may last two months or more (Moyse, 1987). In a few instances, the number of instars is reduced, and the larvae are brooded in the adult or released to develop lecithotrophically (Batham, 1945; Anderson, 1965, 1986; Henry and McLaughlin, 1967; Newman and Ross, 1977; Crisp, 1986). Brooding or reducing the number of larval stages might also be expected in commensal species, but of those few examined, development is planktotrophic and includes the usual complement of larval stages (Moyse, 1961; Molenock and Gomez, 1972; Lang, 1979).

An unusual breeding system has evolved in a number of barnacles including some commensal species. It involves a special case of gregarious settlement wherein small, solely male individuals attach directly to full-sized hermaphrodites (see Newman, 1996). These males function as sperm donors and attach at specific locations or in special niches on the hermaphrodite shell. They generally remain tiny and in some species live only a short time. Darwin (1852, 1854), the first to note these small individuals, called them "complemental males" when associated with hermaphrodites and "dwarf males" when associated with females in dioecious species. Dwarf and complemental males are both found among the stalked scalpellids (Newman, 1996). Among the acorn barnacles, only complemental males are known and these from just a few species (Henry and McLaughlin, 1967; McLaughlin and Henry, 1972; Gomez, 1975; Crisp, 1983; Foster, 1983). In the genus *Chelonibia*, they have been reported in *C. patula* (Ranzani, 1890), which colonizes swimming crabs (Crisp, 1983), and the turtle barnacle *C. testudinaria* (noted in a manuscript by Ross *et al*.).

Turtle barnacles are obligate commensals of sea turtles. They belong to a single balanomorph family, the Coronulidae, and include a number of species that are variously specialized for inhabiting turtles. Some grip the skin, others cement to the scutes of the carapace and plastron or to the large scales of the dermis, and one bores into the shell (Monroe and Limpus, 1979). They exhibit high host specificity and rarely occur on anything except sea turtles and some only on certain turtle species (for exceptions see Edmondson, 1946; Monroe and Garrett, 1979; Chen, 1989; Seigel, 1983).

Chelonibia testudinaria (Linnaeus, 1758) is perhaps the most cosmopolitan of turtle barnacles and the largest, reaching a diameter of 120 mm (Loza and Lopez-Jurado, in press). It cements to host carapace and plastron and is occasionally found on scales of the head and flippers. It is known to associate with all seven species of marine turtles including the flatback (Monroe and Limpus, 1979) and dermis-covered leatherback (Rees and Walker, 1993). Pillai (1956) made the first attempt to study its development, following it to a third stage nauplius. *Chelonibia patula* is the species nearest to a turtle barnacle in which development has been fully documented (Lang, 1979). It is typical in having six naupliar stages followed by a cyprid. The objectives of the present study were to examine reproductive characteristics in *C. testudinaria*, determine whether it undergoes typical larval development, describe its developmental stages, and elaborate on its complemental male.

Materials and Methods

Living specimens of *Chelonibia testudinaria* were collected from green sea turtles, *Chelonia mydas* (Linnaeus, 1758), under the direction and supervision of the marine turtle research group at the Honolulu Laboratory, Pacific Islands Fisheries Science Center, National Marine Fisheries Service (NMFS). The turtles were either stranded and under veterinary care or caught in the field, sampled, and then released. All turtles were from the island of Oahu, Hawaii, mostly from Kaneohe Bay (Lat. 21° 25–31′N, Long. 157° 45–52′W). Following NMFS protocols, barnacles were removed by gently prying them from the turtles' scutes with a thin blade without injuring the host. Gravid individuals were placed in seawater and kept chilled or shaded during transport.

In the laboratory, eggs and larvae were withdrawn from the mantle cavity of the barnacles by pipette and placed in 0.22 μm filtered sea water (FSW) treated with antibiotics (60 μg/mL penicillin and 50 μg/mL streptomycin). Instantaneous fecundity was estimated in several individuals by dispersing the larvae from each in a set volume of seawater and averaging counts from three subsamples. Nauplii were counted instead of eggs, as the latter were strongly adhesive and clung tenaciously to each other, making counting difficult and destructive. The relationship between size and fecundity was assessed by regressing the total numbers of larvae against adult shell volume. Shell volume was estimated using the following equation for an oval-based cone:

$$\text{Volume} = \frac{(L \times W \times 0.8) \times H}{3}$$

where L = rostro-carinal diameter, W = maximum lateral width, and H = maximum height measured from a level surface. Only individuals that were regular in shape were used (i.e., symmetrically oval and not eroded apically).

Embryos were reared in 2-liter beakers at a density of 1/mL and kept at 25°C with aeration. Every other day, the water was changed, and food, a laboratory-reared diatom, *Skeletonema costatum* (Greville, 1866), was added at a density of 1.25×10^5 cells/mL. The molting sequence was tracked by placing individual larvae in 2-mL culture wells and keeping them under the same food and temperature regime as the mass cultures except that food additions and water changes were made each day. Each well was examined daily for the presence of exuviae and measurements were taken of each stage with an ocular micrometer under a compound microscope. The various stages were described from observations of living larvae under a stereoscopic microscope and photographs of fixed larvae taken on a compound microscope. The number and type of setae on the cephalic limbs at each stage were described following Newman and Ross (2001).

In many instances, medium- to large-sized barnacles were found with tiny individuals, putative complemental males, settled in the depressions between the shell plates. In *C. testudinaria*, the shell plates spread apart from each other towards the shell apex with alae and radii (marginal extensions) meeting to span the gap between, forming six wedge-shaped depressions. These depressions, which are broad at the shell rim and narrow at the base, are invested with a series of pits of increasing diameter toward the shell rim. Eight or more pits per depression can be found in large specimens. It is within these pits that the tiny individuals were found attached. A number of small individuals was removed and examined; their reproductive state was compared with individuals of comparable size that had settled and grown on glassware in the lab and with small, solitary individuals removed directly from turtles.

Results

Fecundity and Development

After barnacles were removed from host turtles, gravid individuals were readily identified by the presence of egg plaques filling the mantle cavity. Eggs in early stages of development were pale yellow. Mid-stage embryos were rose-colored and possessed an eye. Late-stage embryos were lavender-gray and were developing appendages. Eggs were ovoid and measured 233 μm (SD 9.5) × 164 μm (SD 7.4). Fecundity ranged from approximately 5000 to 15,000 larvae per animal among four variously sized individuals (Table 1) and correlated strongly with shell volume ($r^2 = 0.93$, $P < 0.05$).

Embryos hatched within the mantle cavity at the Nauplius I (NI) stage. Larvae at the Nauplius II (NII) stage were also frequently found in the mantle cavity. In all, six naupliar stages (NI–NVI) followed by a cyprid stage were observed in *Chelonibia testudinaria* (Table 2). The time from hatching to cyprid took nine days. While stages were most easily differentiated by size, the profile of the cephalic shield (Fig. 1) and the morphology of the limbs (Figs. 2–4) were also diagnostic at each stage. Simple setae (lacking setules) were present on the first naupliar stage. With each stage the number of setae increased as well as the number that were plumose (with setules) (Table 3). Setation patterns of the naupliar limbs in *C. testudinaria* compare favorably with those of *C. patula* (Lang, 1979; Newman and Ross, 2001), and both bear the same four types of setae: simple, plumose, plumo-denticulate and cuspidate. Setation of the naupliar antennules is identical in each species, but setation of the antennae and mandibles differs. The numbers and arrangement of setae in these latter appendages at the NVI stage differ in ways that are substantive for congeners. In *C. patula*, there is an additional seta on the exopodite of the antennae and another at the tip of the endopodite. Also, the setae of the endopodite of the antennae in *C. testudinaria* are all plumose except one that is simple; whereas, in *C. patula*, four of the antennular setae are simple and one is cuspidate. The mandibles differ between the species in setation of the endopodite. Setae at the tip of the endopodite in *C. testudinaria* are simple, whereas in *C. patula*, they are plumo-denticulate. In the adjacent series of setae of the endopodite, there are two simple and two plumose setae in *C. patula* but only two plumo-denticulate setae in *C. testudinaria*. The next cluster of mandibular setae in both species includes two setae that are plumose and one that

Table 1. Fecundity in four *Chelonibia testudinaria* relative to shell size.

	Shell Dimensions (mm)				
Specimen	Length	Width	Height	Volume (mm^3)	Nauplii
1	22.30	19.95	6.70	794.86	4,817
2	27.30	23.75	7.25	1,253.53	8,823
3	27.55	20.20	9.20	1,365.32	11,453
4	28.10	24.60	8.35	1,539.22	15,330

Table 2. Size of naupliar stages in *Chelonibia testudinaria* measured as length and width of the cephalic shield including dorsal thoracic spine.

	Length	Width
Larval Stage	μm (SD)	μm (SD)
NI	242 (28.0)	183 (12.1)
NII	408 (35.4)	249 (19.9)
NIII	496 (11.1)	275 (18.4)
NIV	544 (11.3)	347 (17.9)
NV	630 (27.3)	408 (40.8)
NVI	718 (20.9)	473 (32.3)
cyprid	643 (19.8)	286 (13.1)

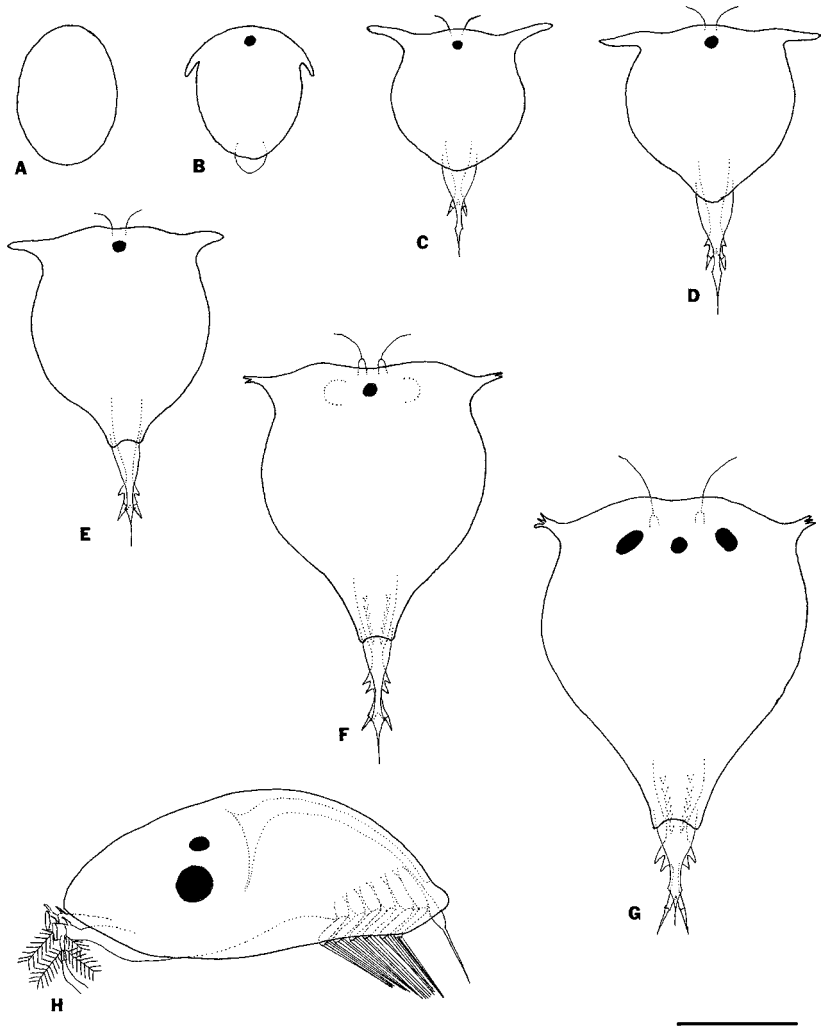

Fig. 1. Larval stages in the development of the barnacle *Chelonibia testudinaria*. A, egg; B–G, dorsal aspect of naupliar stages I–VI showing profile of cephalic shield, dorsal thoracic spine, and trunk; H, cyprid stage. Scale bar = 200 μm.

is cuspidate, whereas in *C. patula*, there is an additional simple seta.

Features of the Larval Stages

Newly hatched NI stage larvae (Fig. 1B) were oval in profile with frontolateral horns that at first were closely adpressed to the sides of the embryo; however, they soon extended out laterally and posteriorly. The frontal margin of the cephalic shield was rounded and arced smoothly down to the tips of the frontal horns. The cephalic shield was also rounded posteriorly. A dorsal thoracic spine was lacking, though a slightly rounded trunk was present. It inserted ventrally, and late in the NI stage it extended beyond the posterior margin of the cephalic shield. This NI stage lacked frontal filaments but did possess a single medial eye, which remained throughout the following naupliar stages. Swimming and feeding appendages were present as a pair of uniramous anterior antennules, a pair of biramous antennae, and a pair of biramous mandibles. All appendages bore long simple setae (Figs. 2A, 3A, 4A; Table 3).

By the NII stage, the frontolateral horns extended out laterally and were approximately even with the frontal margin of the cephalic shield, which exhibited a slight bulge medially (Fig. 1C). A pair of frontal filaments, which appeared at this stage, extended beyond the frontal margin and originated from the ventral

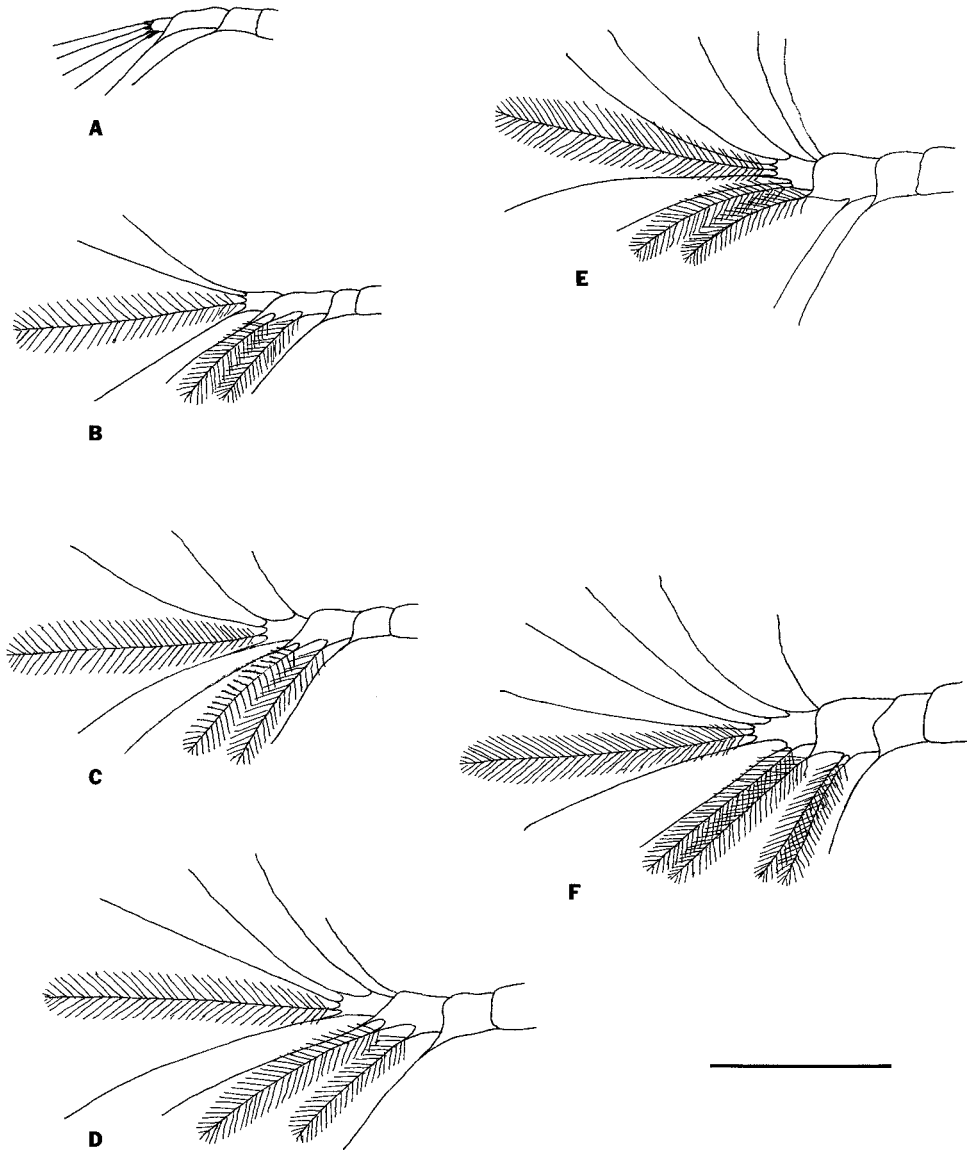

Fig. 2. Right antennule of the six naupliar stages of *Chelonibia testudinaria*. A–F, stages NI–NVI. Scale bar = 200 μm.

surface. Posteriorly the cephalic shield remained rounded, but laterally it widened. A dorsal thoracic spine appeared at this stage, pointing posteriorly. It had a jointed attachment point beneath the dorsal surface of the cephalic shield and could flex ventrally. At this stage, the trunk lengthened and developed a furca at its tip but remained shorter than the dorsal thoracic spine. The first plumose setae of the limbs appeared at this stage (Figs. 2B, 3B, 4B; Table 3).

At the NIII stage, the frontolateral horns remained well extended but appeared proportionately shorter as the cephalic shield continued to widen (Fig. 1D). The frontal margin of the cephalic shield remained relatively straight, while the posterior margin developed a blunt tip. The dorsal thoracic spine lengthened and bore a pair of tiny barbs towards its posterior end. The trunk remained shorter in length than the dorsal thoracic spine, and it thickened and developed a pair of ventrally directed spines just anterior to the terminal furca. Limbs continued to add setae and increased in complexity (Figs. 2C, 3C, 4C; Table 3).

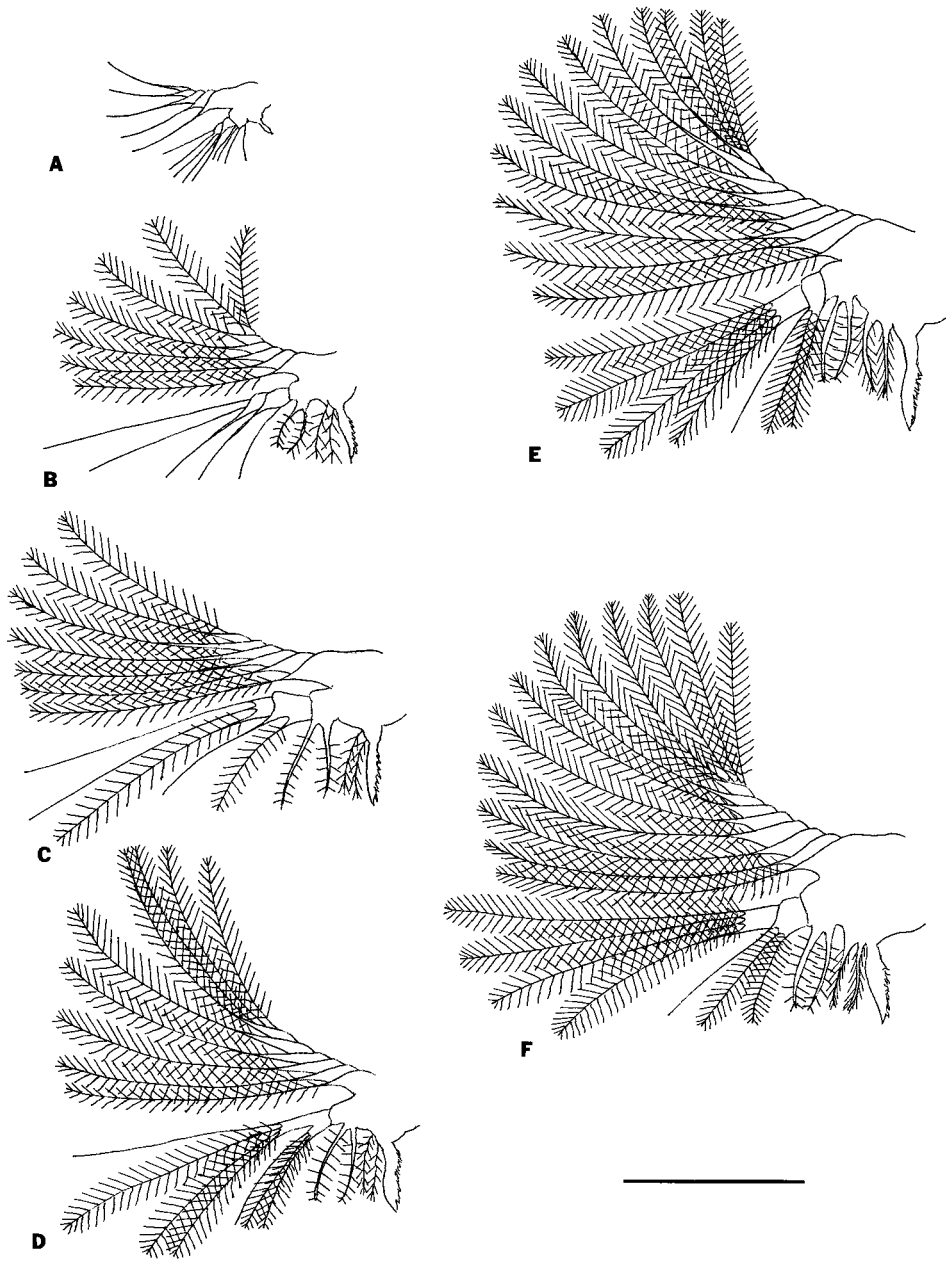

Fig. 3. Right antenna of the six naupliar stages of *Chelonibia testudinaria*. A–F, stages NI–NVI. Scale bar = 200 μm.

Stage NIV nauplii were characterized by frontolateral horns that pointed somewhat ventrally as the surface of the cephalic shield began to arch dorsally (Fig. 1E). The frontal margin of the cephalic shield lost its anterior bulge and became straight or slightly concave. Posteriorly, the cephalic shield margin was no longer blunt-tipped; rather, two projections extended from it along either side at the point of attachment for the dorsal thoracic spine, creating a saddle-shaped posterior notch. The dorsal thoracic spine increased in length and width, and the trunk became approximately equal to it in length. Limbs continued to add setae, and the number that were plumose increased (Figs. 2D, 3D, 4D; Table 3).

By stage NV, the frontolateral horns became relatively short, and their tips were cleft (Fig. 1f). They pointed ventrally as the cephalic

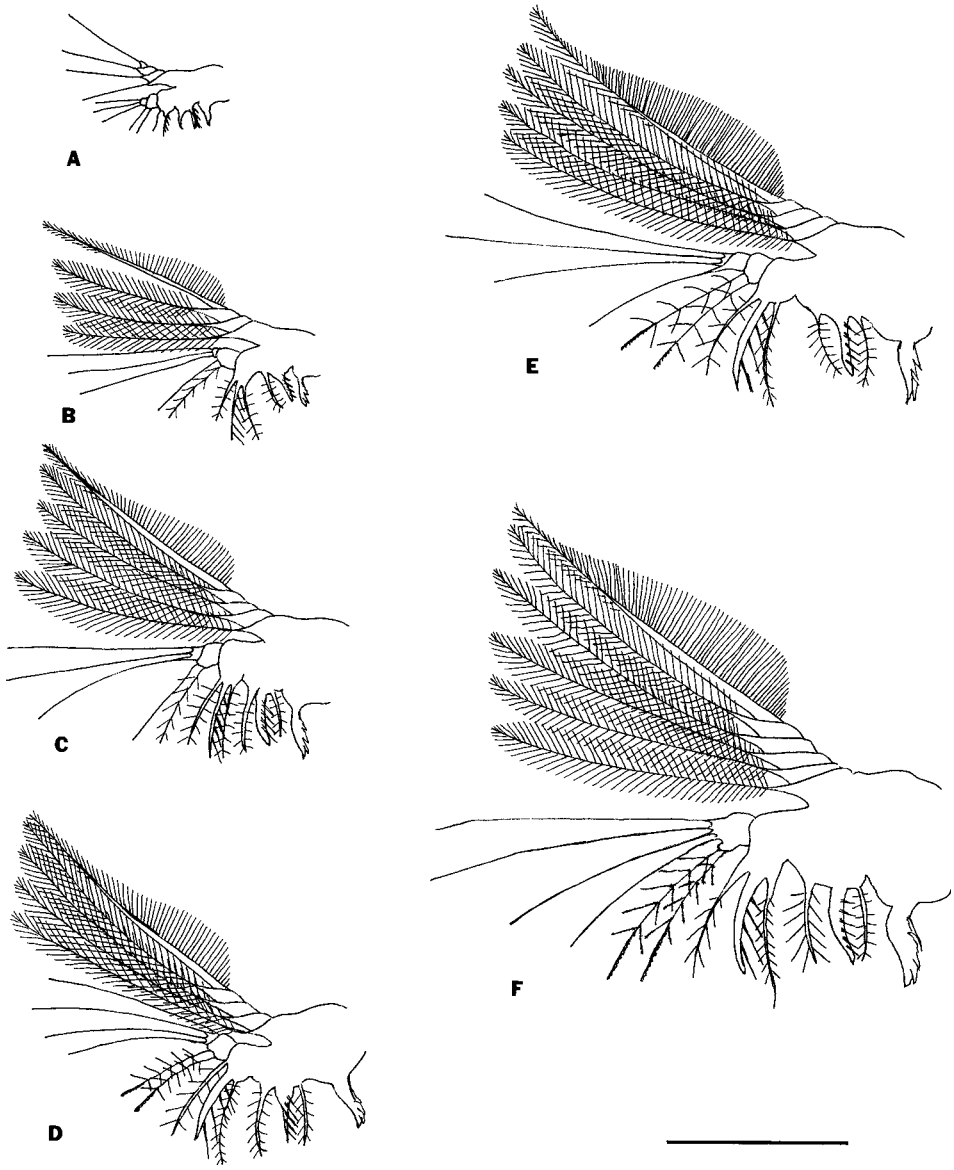

Fig. 4. Right mandible of the six naupliar stages of *Chelonibia testudinaria*. A–F, stages NI–NVI. Scale bar = 200 μm.

shield deepened dorsoventrally. Two incipient compound eyes appeared faintly at this stage, one on either side of the medial eye. The dorsal thoracic spine at this stage was still longer than the trunk. Another pair of ventrally directed spines developed on the trunk, and limb primordia became visible on its ventral surface as six plates. The frontal filaments were seen to insert on a protuberant base. A few setae were added to the antennule and antenna but remained constant on the mandible (Figs. 2E, 3E, 4E; Table 3). The setae of the antenna were nearly all plumose at this stage.

Stage NVI (Fig. 1G), the final naupliar stage, was characterized by strongly cleft frontolateral horns that pointed in the ventral direction. The cephalic shield was strongly arched dorsoventrally. Two well-developed, lateral compound eyes were present in addition to the medial eye. The dorsal thoracic spine was shorter at this point than the thicker and longer trunk, which bore the thoracic limb primordia. An additional

Table 3. Setation formula of the limbs of the naupliar stages for *Chelonibia testudinaria*. Letter codes correspond to setal types of Lang (1979): C, cuspidate: D, plumo-denticulate; G, gnathobase; P, plumose; S, simple. Numerals indicate counts of similar type setae in a series.

		Antenna		Mandible	
Stage	Antennule	Exopodite	Endopodite	Exopodite	Endopodite
NI	. . . SSSS S . S .	S 4S -	. SSS SS . SS SS G	S 3S -	. SSS SS SC . SC . G
NII	. . . SSPS SP P S . .	P 6P -	. SSS SS . PP PP G	P 3P -	. SSS SP PCP PC . G
NIII	. . S SSPS SP P S . .	P 6P -	. SSP SP . PP PP G	P 4P -	. SSS SP PCP PCP G
NIV	. S S SSPS SP P S . .	P 8P -	SPPP SPP PP PP G	P 4P -	SSSS DD PCP PCP G
NV	S S S SSPS SP P S . S	P 11P -	PPPP SPP PP PP G	P 4P -	SSSS DD PCP PCP G
NVI	S S S SSPS SP P P P S	P 11P -	PPPP SPP PP PP G	P 5P -	SSSS DD PCP PCP G

seta was added to both the antennule and mandible at this stage, while the number of setae of the antenna were constant (Figs. 2F, 3F, 4F; Table 3).

The terminal larval stage, the cyprid (Fig. 1H), was narrower and longer in profile than the preceding stage. It bore a medial naupliar eye and two compound eyes. The cephalic shield had become a bivalved carapace, and the caudal furca and setae of the thoracic limbs protruded posteroventrally. The six thoracic segments each bore a pair of natatory limbs. Anteriorly, the antennules each bore a cup-shaped attachment organ with a finely setose ramus.

Juveniles and Complemental Males

In mass culture, many larvae settled and metamorphosed into juveniles on the sides of the glass beakers within one to two days of reaching the cyprid stage. A number of these juveniles was maintained in culture and observed for more than 200 days. They were kept aerated at room temperature and were fed undetermined quantities of the diatom *Skeletonema costatum* and the flagellate *Isochrysis galbana* Parke, 1938. Juveniles began calcifying the shell shortly after settlement. Darwin (1854) noted that *C. testudinaria* gives the impression of having a six-plated shell because the rostrolateral plates join tightly with the rostral plate. The actual eight compartments of the shell were observed in initial shell development (Fig. 5A), but by 12 to 24 hours the coalescence of the above mentioned plates could already be seen (Fig. 5B).

Reproductive states compared between juveniles settled on glassware in the laboratory and solitary individuals growing on turtles differed markedly from the putative complemental males attached to the shells of hermaphrodites (Table 4). Though comparable in size to the laboratory-settled animals and a little smaller than the turtle-attached individuals, the individuals associated with hermaphrodites had large penises and active sperm, whereas the others lacked any sign of reproductive development including a penis. The average maximum basal diameter of four small males attached to a hermaphrodite measured 6.6 mm (SD 0.6) with an average penis length of 4.5 mm (SD 0.9) when preserved. The average copulation distance (measured from the center of each small male to the center of the hermaphrodite) was 9.3 mm (SD 2.0). Given that in life, an extended penis may be four to five times its contracted length (Crisp, 1983), these males were well within range to copulate with the hermaphrodite. When attached to a hermaphrodite, individuals as small as 2.2 mm maximum basal diameter were found with penises.

Discussion

This is the first study of a barnacle commensal with sea turtles in which all larval stages have been documented; it also marks another case of androdioecious breeding in the genus *Chelonibia*. The turtle barnacle *Chelonibia testudinaria* undergoes typical cirriped development involving seven larval stages. First-stage nauplii hatch from eggs within the mantle cavity of the adult and are brooded through the first molt to second-stage nauplii. They are then released into the plankton, where they feed and develop to a sixth naupliar stage. This stage is followed by the nonfeeding cyprid, which searches out and attaches to a suitable host. The developmental period in *C. testudinaria* is nine days in length, which compares favorably with a barnacle epizoic on gorgonians reared at the same temperature (Molenock and Gomez, 1972) and *Balanus amphitrite* Darwin, 1854, a cosmopolitan warm-water species (Costlow and Bookhout 1958; Anil *et al*., 2001). How long *C. testudinaria* remains competent to settle once it

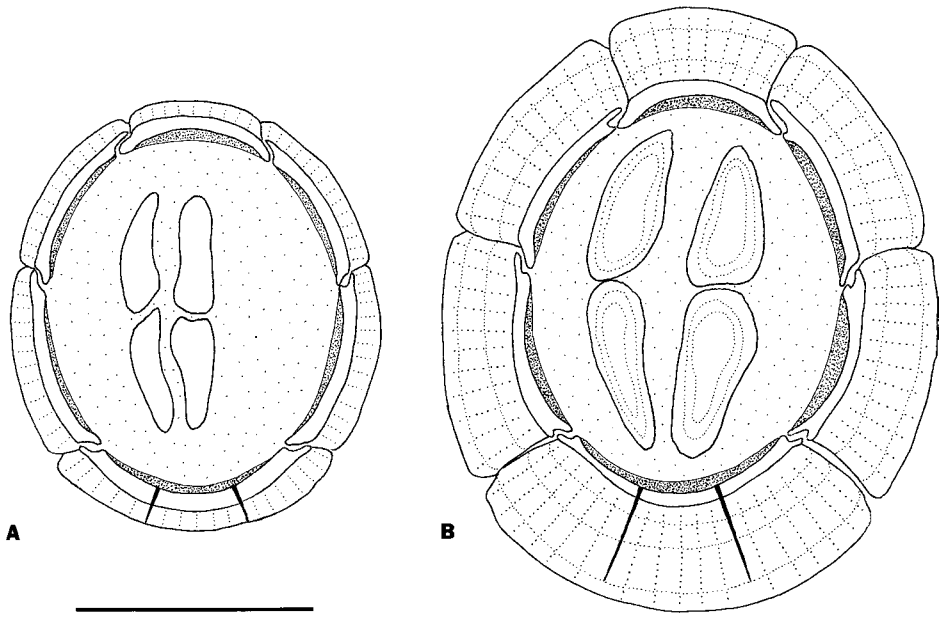

Fig. 5. Shell growth in juvenile *Chelonibia testudinaria*. A, Several hours post-settlement, showing the secretion of the eight parietal compartments. B, Two days post-settlement, suture lines between the eight parietal plates are distinct but the carino-laterals are beginning to coalesce with the carina to give the appearance of six compartments. Scale bar = 400 μm.

reaches the cyprid stage is unknown. That it settles on laboratory glassware within one or two days of completing development possibly indicates that it does not delay settlement for long; however, a range of two to four weeks is not unusual for other barnacles (Lucus *et al*., 1979; Anderson, 1994). Its breeding cycle and frequency of reproduction remain unknown. The life span for this species is also not known; however, as it adheres only to the outer surface of the host shell, the scute, it must be lost with the molting of the outer shell layer that occurs approximately every year (Monroe, 1981). Molting is a piece-meal event, at least in Hawaiian green turtles, with scute material sloughing off in bits and flakes rather than in sheets (G. H. Balazs, personal communication). Barnacles likely hasten this process as they grow larger and increase drag.

Sea turtles host a variety of epizoites including a number of opportunistic barnacle species (Green, 1998; Frick and Slay, 2000). How some barnacle species have become obligate associates of these mobile hosts remains an interesting question. Barnacle–turtle associations have likely been evolving for tens of millions of years. Modern-day families of sea turtles can be traced back as far as the Cretaceous (Pritchard, 1997), and *Chelonibia* is known from the Oligocene (Zullo, 1982). Today, a dozen or more commensal barnacles occur with sea turtles (Monroe and Limpus, 1979).

For barnacles to succeed as obligate commensals, their host must occupy an area at appreciable densities and for sufficient lengths of time to allow barnacle larvae time to emerge, develop in the plankton, and recruit in adequate abundance for reproduction. The most parsimonious scenario for turtle barnacles is that they recruit to hosts primarily near shore where turtles aggregate to feed or mate. It may be primarily older turtles that are colonized as pre-reproductive turtles typically remain at sea for many years (Balazs, 1995; Miller, 1997) and may thus evade colonization by barnacles. Evidence supporting this near-shore recruitment hypothesis comes from observations that turtles that have been at sea for extended periods are devoid of barnacles (C. J. Limpus and G. H. Balazs, personal communication). Also, loggerhead turtles nesting in Georgia have been found to have a higher occurrence of coastal barnacle species than pelagic barnacle species (Frick *et al*., 1998). A recent phylogeographic study of *C. testudinaria* on loggerhead turtles also supports this hypothesis (Rawson *et al*., 2003). From our

Table 4. Male development in small *Chelonibia testudinaria* compared by settlement substrata: solitary individuals on laboratory glassware, individuals in settlement pits on "host" hermaphrodites, and solitary individuals on sea turles.

		Length (mm)		Width (mm)		
Substratum	Age (days)	Avg.	(SD)	Avg.	(SD)	Penis
Glass ($n = 10$)	226 ± 2	3.4	(0.2)	2.9	(0.3)	absent
Barnacle ($n = 7$)	unknown	4.0	(0.8)	3.2	(0.9)	present
Turtle ($n = 6$)	unknown	7.8	(1.8)	6.7	(1.5)	absent

limited sampling in Hawaii, the incidence of epizoic barnacles was highest on green turtles resident in Kaneohe Bay, Oahu. This bay, which is protected by a fringing reef that may help retain larvae, is a common feeding ground for juvenile and adult green sea turtles.

Current population levels of sea turtles make it difficult to conceive of them as a dependable substratum for barnacles; however, sea turtles may have been much more abundant in the past. Population estimates from harvesting records and estimates of herbivore carrying capacity put historical levels of green turtles at tens to hundreds of millions in the Caribbean Sea alone (Jackson, 2001; Bjorndal and Jackson, 2003). Declines in the abundance of sea turtles due to hunting, egg harvesting, and habitat loss may also impact the barnacles that rely on them as hosts. Decreased turtle numbers undoubtedly limit turtle-barnacle populations and could lead to the extinction of some species.

The dynamics of barnacle recruitment on sea turtles are as yet unexamined, but chemical signaling from host to commensal may be a key element. A number of invertebrates are known in which chemical cues from host, prey, or biogenic substrata guide settlement and induce metamorphosis (Hadfield, 1977; Pawlik, 1992; Hadfield *et al*., 1994; Clare, 1995; Rittschof *et al*., 1998; Hadfield and Paul, 2001). Intertidal barnacles, in particular, are known to respond to a protein cue from conspecifics that induces settlement when adsorbed to surfaces (Crisp and Meadows, 1962, 1963). The role of chemical cues has been implicated (Molenock and Gomez, 1972) but never directly tested in the settlement of commensal barnacles.

The relatively high reproductive output we found in *C. testudinaria* is proportional to its size and is likely necessary to ensure its successful colonization of turtles. Whether it breeds seasonally or year round is not known. We found individuals that were reproductive from spring to fall. The species likely produces broods in quick succession as do other barnacles (Anderson, 1994). Its rate of reproduction is undoubtedly facilitated by a breeding system involving small complemental males that produce sperm at a young age.

The location in which the complemental males of *C. testudinaria* attach is unique among barnacles. We found them outside the orifice of the hermaphrodite shell in the depressions between the shell plates. Males occupy recesses or niches within these depressions, perhaps specialized for their settlement. In *C. patula*, complemental males are found at the margin of the orifice of the hermaphrodite (Crisp, 1983), at the apex of each shell wall rather than in the depressions between. Because of their settlement location at the rim of the orifice, complemental males in *C. patula* have been termed "apertural males" (Crisp, 1983); though, more correctly they are "orificial males" (*sensu* Ross *et al*., MS). This terminology does not justly apply to the males of *C. testudinaria* as they attach away from the hermaphrodite orifice, yet we stop short of erecting a new term for them.

Hermaphrodites of relatively large size have been found in *C. patula* inhabiting the same location as complemental males, suggesting that males in this species are likely protandric individuals arrested or slowed in growth (Crisp, 1983). In *C. testudinaria*, we found complemental males that were beginning to outgrow the depressions in which they had settled (Fig. 6B); however, none were ever found that had attained any great size or that were developing female characteristics.

It is common for several complemental males to occur on a single hermaphrodite and for multiple males to be found within a single depression in *C. testudinaria*. Those attached nearer the rim, where the depressions are wider and the settlement pits are larger, presumably have the advantage of growing larger or faster and of having greater opportunity to copulate with their "host" hermaphrodite. However, we commonly found males attached away from the opercular rim. This may be due to preferential

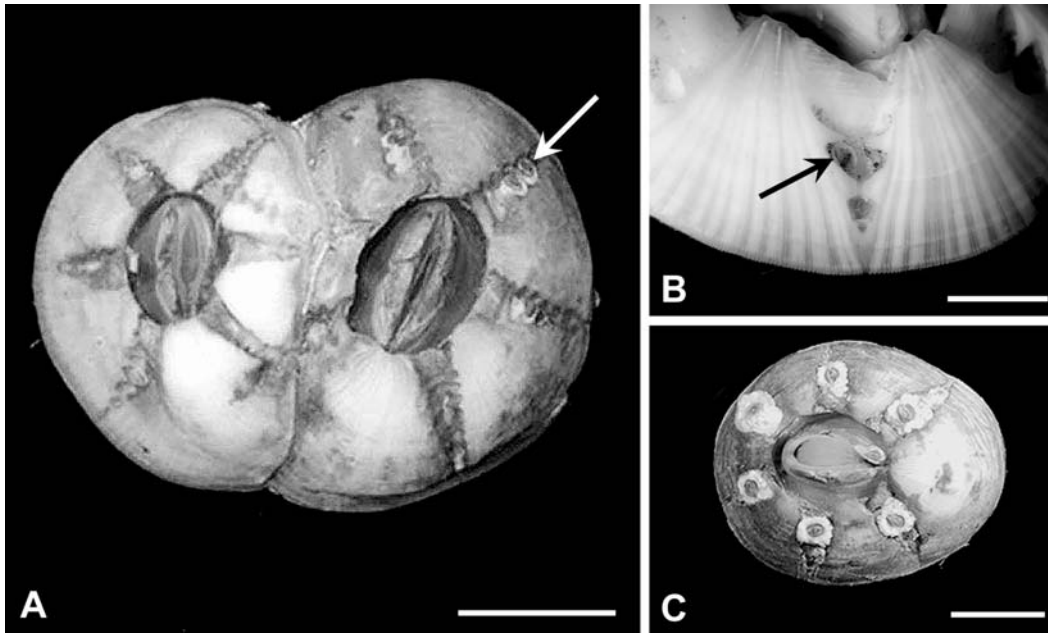

Fig. 6. Attachment locations of complemental males in *Chelonibia testudinaria*. A, Two hermaphrodites joined at their bases display depressions between wall plates invested with settlement pits in which complemental males (white arrow) are attached. Scale bar = 10 mm. B, Settlement pit formation in a carino-lateral shell depression of a young hermaphrodite and a recently settled juvenile (black arrow). Scale bar = 2 mm. C, Later-stage complemental males overgrowing the settlement pits and shell depressions of a hermaphrodite and one male attached to its scutum. Scale bar = 10 mm.

settlement by larvae or removal of males near the orifice. The summits of large "host" hermaphrodite shells are smoothly domed, and males attached near the orifice may be subject to greater amounts of abrasion and higher rates of dislodgment. Males nearest the orifice may also represent old, perhaps "spent" individuals that die and fall off easily.

Complemental males are unusual among acorn barnacles and across the Cirripedia, and males (both dwarf and complemental) vary considerably in form and function (Klepal, 1987). Foster (1983) described three classes of complemental males: "Those that have trophi and are essentially minute forms of their associate hermaphrodites ... [t]hose with degenerate trophi and occurring in special pouches near the orifice of their 'host' [and] ... [t]hose of *Ibla* spp. that reside inside the mantle cavity, with degenerate trophi or without trophi and then either attached or lying loosely respectively." Males of *C. testudinaria* and *C. patula* fit the first designation, except for *C. testudinaria*, which arguably settles in special "pouches" near, but outside, the orifice. The genus *Chelonibia* is composed of four extant members, all obligate commensals (*C. testudinaria*, *C. patula*, *C. caretta* (Spengler, 1790) and *C. manati* (Gruvel, 1903); it is unknown whether complemental males occur in the latter two species. Diversity within the genus may actually be higher, as several subspecies are recognized in *C. manati* (Pilsbry, 1916), a commensal of manatees (Mignucci-Giannoni *et al*., 1999) and cryptic species may be present in *C. testudinaria* (Rawson *et al*., 2003). We have examined specimens of *C. caretta* from Florida for small males without success. The shell in this species is smaller and less dome-shaped than *C. testudinaria*, and it does not form wide depressions between the wall plates. Individuals tend to cluster together at their bases, and we have not seen small individuals attached near the shell apex.

Complemental males are an advantage to commensal species when population densities are low and when large hermaphrodites settle out of copulation range of each other. These males also allow for an earlier time to reproduction, reducing generation time (Foster, 1983). Multiple males on a large hermaphrodite may also increase genetic exchange through

compound inseminations (Crisp, 1983). In *C. testudinaria*, which attains a large size, small males could also be important in reducing the weight and hydrodynamic drag of barnacle aggregations, prolonging the time before they are lost to host molting. We are continuing our studies into the biology of *C. testudinaria*, testing its degree of settlement selectivity and characterizing its phylogeographic patterns across host boundaries.

Acknowledgements

We are indebted to friends and colleagues who assisted in this work. Foremost, George Balazs and his marine turtle research group at the Honolulu Laboratory of the Pacific Islands Fisheries Science Center, National Marine Fisheries Service, was instrumental in making this study possible. He and his dedicated team provided the critical link in procuring barnacles from sea turtles and most importantly, in obtaining them live. Cecelia Miles provided access to her photographic microscope system. Brian Nedved, Brenden Holland, and Anuschka Faucci provided valuable discussion and technical support. Vanessa Fread helped in the rearing of phytoplankton and larvae. Comments and corrections by two anonymous reviewers are gratefully acknowledged. This work was supported by funding to MGH from the Office of Naval Research (grant No. N00014-03-1-0078).

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RECEIVED: 21 November 2003.
ACCEPTED: 22 April 2004.