

Development of the hectocotylus in *Illex coindetii* (Verany, 1837) (Cephalopoda: Ommastrephidae)

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SUMMARY: Species of the genus *Illex* (Family Ommastrephidae) are distinguished morphologically mainly based on differences in the hectocotylus, the modified arm that males of many cephalopod species use to transfer spermatophores to females during mating. We examined the developmental details of the hectocotylus in *Illex coindetii*, the species of *Illex* with the most highly modified hectocotylized arm, to determine whether it has an ontogeny that passes through stages that are similar to the less modified hectocotyls of sympatric *I. illecebrosus* and *I. oxygonius*. The development of modifications of one of the ventral arms is essentially synchronous on all portions. The hectocotylus of *I. coindetii* is therefore morphologically distinctive even at early maturity stages, and therefore should not be easily confused with that of its sympatric congeners.

Keywords: squid, development, maturity, arm modification, identification, taxonomic character.

RESUMEN: DESARROLLO DE LA HECTOCOTYLUS EN *ILLEX COINDETHI* (VERANY, 1837) (CEPHALOPODA: OMMASTREPHIDAE). – Las especies del género *Illex* (Familia Ommastrephidae) se distinguen morfológicamente principalmente por las diferencias en el hectocotylus, el brazo modificado que los machos de muchas especies de cefalópodos utiliza para transferir espermatóforos a las hembras durante el apareamiento. Hemos examinado los detalles del desarrollo de la hectocotylus en *Illex coindetii* la especie de calamar *Illex* con el brazo hectocotylizado más altamente modificado, porque podría haber una ontogenia que pasa a través de etapas que son similares a los menos modificados hectocotyls de simpátricas *I. illecebrosus* y *I. oxygonius*. El desarrollo de las modificaciones de uno de los brazos ventrales es esencialmente sincrónico en todas las partes. El hectocotylus de *I. coindetii* es por lo tanto morfológicamente distintivo, incluso en estados de madurez temprana y por lo tanto no debe ser fácilmente confundida con la de sus congéneres simpátricas.

Palabras clave: calamar, desarrollo, madurez, modificación del brazo, identificación, carácter taxonómico.

INTRODUCTION

The hectocotylus is a modified arm on males of many cephalopod species. It is used to transfer spermatophores to females during mating. Hectocotylation occurs in all ommastrephid species, and the characteristics of the hectocotylus are important for identifying species as well as evaluating phylogenetic relationships among the members of the Ommastrephi-

dae (Roeleveld 1988). Species within the commercially important genus *Illex* are differentiated based largely on hectocotylus morphology (Roper *et al.* 1998, Martinez *et al.* 2002). This can cause difficulties in identifying specimens that are not mature males and has contributed to controversy about how many species of *Illex* actually exist.

Several authors (e.g. Roper *et al.* 1969, Roeleveld, 1988) consider the genus *Illex* to comprise four spe-

cies: *Illex illecebrosus* (Lesueur 1821), *Illex coindetii* (Verany 1839), *Illex argentinus* (de Castellanos 1960), and *Illex oxygonius* Roper, Lu and Mangold 1969. However, some authors only recognize two species: *Illex illecebrosus* (including three subspecies *illecebrosus*, *coindetii* and *oxygonius*) and *Illex argentinus* (Zuev 1966, Nesis 1987). A third concept considers only three species to be valid, with *I. oxygonius* thought to be the mature male of *I. illecebrosus* (Nigmatullin 1992, Lipinski pers. comm.).

As in many squid species, hectocotylyzation in the *Illex* species consists of modifications to either of the ventral arms. The hectocotyli of all *Illex* species share a basic structure: modification of the tip of the hectocotylyzed arm, the true hectocotylus, characterized by transformation of sucker stalks into lamellae, knobs and papillae, and changes in size and dentition of some suckers. No signs of hectocotylyzation develop on the opposite arm. The non-hectocotylyzed arm is shorter than the hectocotylyzed arm in all *Illex* species except *I. illecebrosus*, in which the opposite arm is the longest of the pair. The ratio of the length of the hectocotylus tip to the total length of the hectocotylyzed arm is 22% in *Illex illecebrosus*, 25% in *I. coindetii*, 29% in *I. oxygonius*, and 50% in *I. argentinus* (Lu 1973).

The hectocotylus of *I. coindetii* is unique because, distal to the 7th pair of suckers (counting from their proximal origin on the arm), the trabeculae (conical appendages of the sucker stalk bases) in the medial part of the arm are transformed into papillose fringed flaps (Lu 1973). Four regions are recognized in the hectocotylus of *I. coindetii*: the suckerless basal part of the arm, the proximal and medial regions with suckers and fringed flaps, and the distal hectocotylyzed tip (Fig. 1). The suckers on the proximal-most region are similar to those of the other seven arms, whereas the suckers on the medial part are smaller in size. The interface between the proximal and medial regions is marked by a constriction in the arm musculature (Roper and Mangold 1998). The dentition of the sucker rings differs between the proximal and medial regions of the arm (Naef 1923). The suckers of the proximal part have normal dentition with a central round tooth and three to five smooth rounded lateral teeth, whereas teeth in the smaller suckers of the medial part are sharper and longer. Zecchini *et al.* (1996) quantitatively compared hectocotylus morphology in *I. coindetii* populations from the Tyrrhenian Sea and the Gulf of Mexico and found only minor differences that were insufficient for taxonomic separation.

Very little research has been published on the ontogeny of the hectocotylus. Steenstrup (1857) provided some observations in his anatomical review of the hectocotylus in cephalopods. The main ontogenic discovery from that work is that the modifications on the tip of the hectocotylus in squid derive from the sucker stalks and bases.

There are four possible ways that the modifications of the hectocotylyzed arm in *Illex* species could devel-

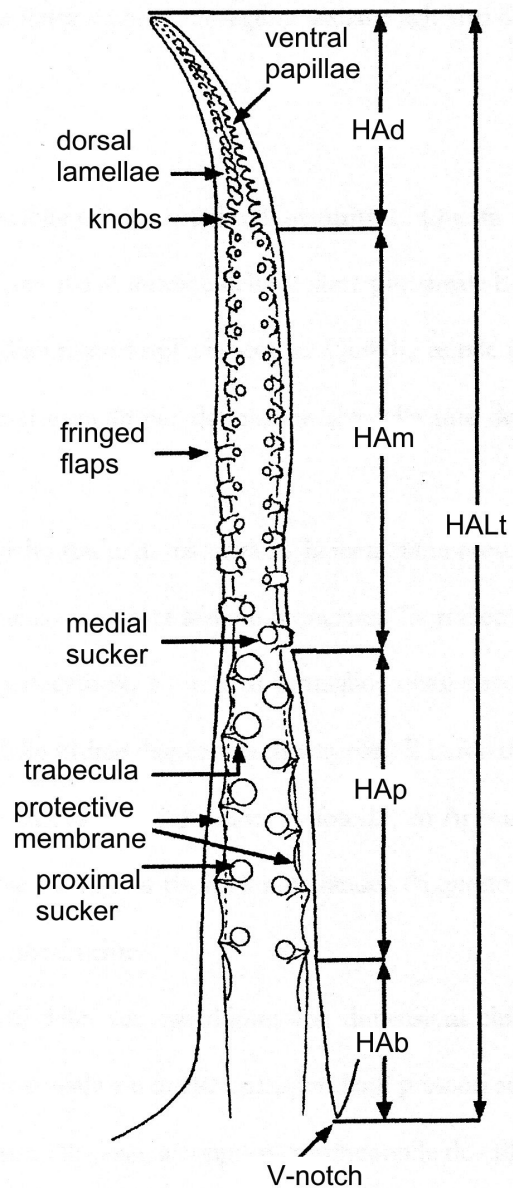


FIG. 1. – Diagrammatic illustration of hectocotylyzed left ventral arm of *Illex coindetii*. Definitions given in Appendix 2.

op: (1) from the distal end to the proximal end, (2) from the proximal end to the distal end, (3) from a medial area towards both the proximal and distal ends, or (4) synchronously throughout its length. In the first case, all species would develop through an initial stage in which the developing hectocotylus is characterized by modifications to only a small percentage of the distal tip, as in *I. illecebrosus*.

Three species, *I. coindetii*, *I. illecebrosus* and *I. oxygonius*, are sympatric in the western North Atlantic. Under some potential developmental scenarios, it would not be possible to confidently identify males from this region that are not fully mature to species level. The hectocotylus of *Illex coindetii* is the most highly modified of the three sympatric species in the

North Atlantic because its medial region is secondarily modified. If this secondary modification occurs late in ontogeny, the hectocotylus of *I. coindetii* may develop through an *I. illecebrosus*-like stage. So, if common stages exist among species, at which point during development does the hectocotylus become a reliable taxonomic character? Both large and small males of *I. illecebrosus* and *I. coindetii* have been found to be fully mature. What, therefore, is the relationship between size, maturity stage, and hectocotylus development among *Illex* species?

Arguments about hectocotylus development based on specimens identified based on hectocotylus morphology could be the result of circular reasoning. Therefore, to address the questions mentioned above, we collected male specimens of *I. coindetii* from the Mediterranean Sea, because this is the only *Illex* species present in these waters. This eliminates the potential identification problems involved when there are sympatric species.

MATERIALS AND METHODS

Sixty specimens of *Illex coindetii*, 35-125 mm ML, were obtained from commercial catches landed in November 1992 and May 1993 at Porto St. Stefano (Grosseto, Italy) in the northern Tyrrhenian Sea. They were fixed in 8% formalin and preserved in 45% isopropanol as recommended by Roper and Sweeney (1983). Animals at all maturity stages, the smallest of which were around 75 mm mantle length (ML), were compared to determine how the hectocotylus develops as the animals mature.

Due to possible artifacts in weight or differential shrinkage resulting from preservation, we decided not to assess maturity using scales based on weight indices as in Vovk (1972) or length indices involving different tissue types, such as Macy's (1982) index. Instead we used a modification of Lipinski's scale (Lipinski 1979, Lipinski and Underhill 1995). This is a simplified maturity scale that comprises six stages, including the spent stage (Appendix 1).

Whenever possible, the abbreviations used (Appendix 2) were based on or modified from recommendations made in Roper and Voss (1983). Additional measurements, counts and indices not present in that work are introduced herein. For example, the non-hectocotylized ventral arm is termed the "opposite arm". Some of the abbreviations used are shown in Figure 1. All measurements are in mm.

The diameter of the suckers and number of minute suckers on the tip of the hectocotylus were assessed using a dissecting microscope with ocular micrometer. Fine details of lamellae, papillae, fringed flaps and sucker dentition were examined with scanning electron microscopy (S.E.M.). A critical point dryer was used for S.E.M. samples following dehydration in a graded series of ethanol concentrations (70%, 80%, 95% - double immersion, and 99%). Once dried, the

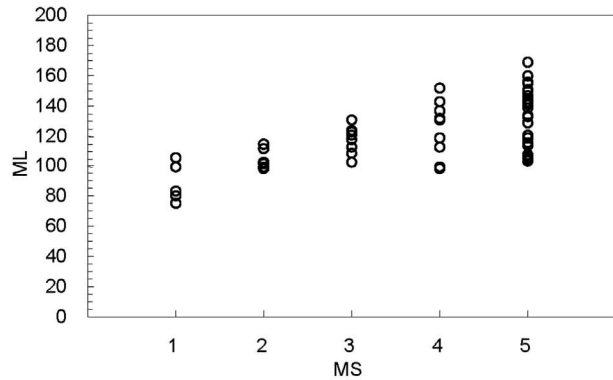


FIG. 2. – Plot of mantle length (ML, in mm) vs maturity stage.

samples were coated with carbon and gold. If a sucker was large enough, the chitinous ring was removed from the muscular cup in order to improve the visibility of the dentition; it is easier to mount the isolated chitinous ring on an S.E.M. stub for coating. Occasionally the muscular tissue around the chitinous ring was loosened by putting the sucker in a very dilute solution of KOH for 1 to 2 minutes. The dentition on very small suckers was determined with the suckers still attached to a portion of the arm so as not to damage them.

All spermatophores in the Needham's sac of ten fully mature males were counted and measured to determine possible relationships between hectocotylus measurements and the spermatophore length.

The statistical analyses were selected based on the nature of the variables. When an independent variable was continuous (e.g., ML), linear regression was performed. For discrete independent variables like maturity stage, we used the Tukey-Kramer method for comparing the sample means. This method uses harmonic means and is appropriate when samples of different sizes are being studied (Sokal and Rohlf 1981). The numbers of specimens examined differed among maturity stages based on the availability of specimens.

RESULTS

Mantle length and maturity stages

The relationship between mantle length (ML) and maturity stage (MS) was positive but highly variable (Fig. 2). Some specimens as large as 106 mm ML were immature, whereas some fully mature squid were as small as 104 mm ML. All specimens larger than 131 mm ML were either early mature or fully mature. Statistical tests of differences in mean ML at each MS indicated that mean ML increased significantly with each MS.

Comparison of hectocotylized and opposite arms

Of 60 specimens, 37 were hectocotylized on the right ventral arm and 23 on the left ventral arm. How-

TABLE 1. – Regression equations discussed in the text, with correlation and significance of correlation. Numbers in parentheses are the regression coefficient \pm standard deviation.

HALt=(0.806 \pm 0.060)ML + (20.816 \pm 10.389);	R=0.866; P>0.99
OALt=(0.771 \pm 0.051)ML + (-18.052 \pm 8.533);	R=0.894; P>0.99
HAb=(0.052 \pm 0.016)ML + (2.173 \pm 1.832);	R=0.52; P>0.99
HAp=(0.184 \pm 0.038)ML + (-0.405 \pm 5.466);	R=0.557; P>0.99
HAm=(0.226 \pm 0.025)ML + (1.385 \pm 3.613);	R=0.775; P>0.99
PC=(0.188 \pm 0.056)ML + (-4.402 \pm 6.716);	R=0.480; P>0.99
LC=(0.147 \pm 0.035)HALt + (6.751 \pm 4.163);	R=0.521; P>0.99
HAd=(0.228 \pm 0.035)ML + (-7.940 \pm 4.659);	R=0.686; P>0.99
SDp=(0.015 \pm 0.003)ML + (-0.212 \pm 0.366);	R=0.616; P>0.99
SDp=(0.020 \pm 0.002)HALt + (0.002 \pm 0.289);	R=0.777; P>0.99
HASr=(0.006 \pm 0.001)HALt + (-0.030 \pm 0.150);	R=0.579; P>0.99
HASr=(0.004 \pm 0.001)ML + (0.027 \pm 0.171);	R=0.380; P>0.99
SpL=(0.187 \pm 0.038)ML + (-2.530 \pm 2.201);	R=0.796; P>0.99
SpL=(0.179 \pm 0.069)HALt + (6.598 \pm 2.201);	R=0.568; P>0.95
SpL=(0.524 \pm 0.199)Ham + (5.948 \pm 7.054);	R=0.575; P>0.95
SpL=(0.480 \pm 0.162)HAd + (11.595 \pm 4.392);	R=0.619; P>0.95

TABLE 2. – Average value of difference in length (DL), standard deviation (SD), range and frequency of positive DL during maturity stages (MS) of *Illex coindetii*.

MS	n	DL	SD	Range	f(DL >0)
1	7	-3.3%	3.6%	-9.26% to +2.56%	20.0%
2	7	-5.1%	6.2%	-15.6% to +1.9%	33.3%
3	9	-2.3%	4.7%	-8.0% to +5.7%	25.0%
4	9	-0.1%	4.9%	-7.2% to +6.4%	44.4%
5	28	+5.3%	6.6%	-6.5% to +18.6%	78.6%

ever, these values are not significantly different from the null ratio 1:1 based on the chi-square test ($\chi^2=3.267$, $df=1$). Although the overall percentage of animals in which the hectocotylized arm is longer than the opposite arm (positive difference in length – positive DL)

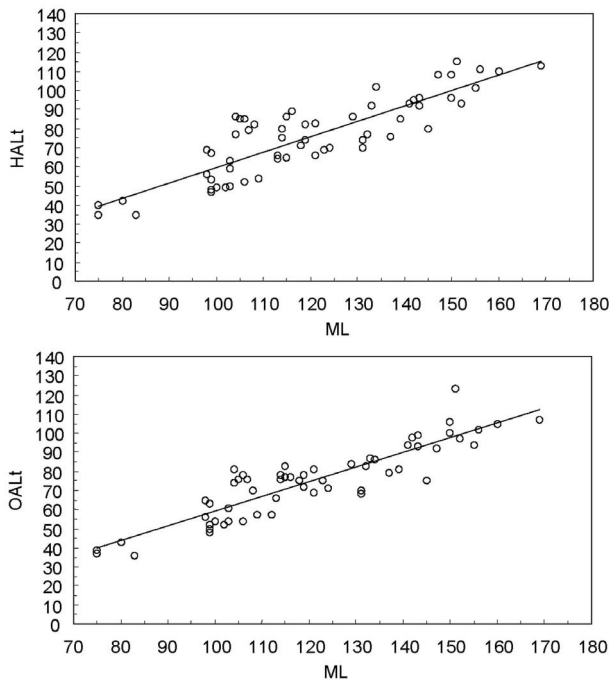


Fig. 3. – Regression lines for HALt and OALt plotted against mantle length. Measurements in mm.

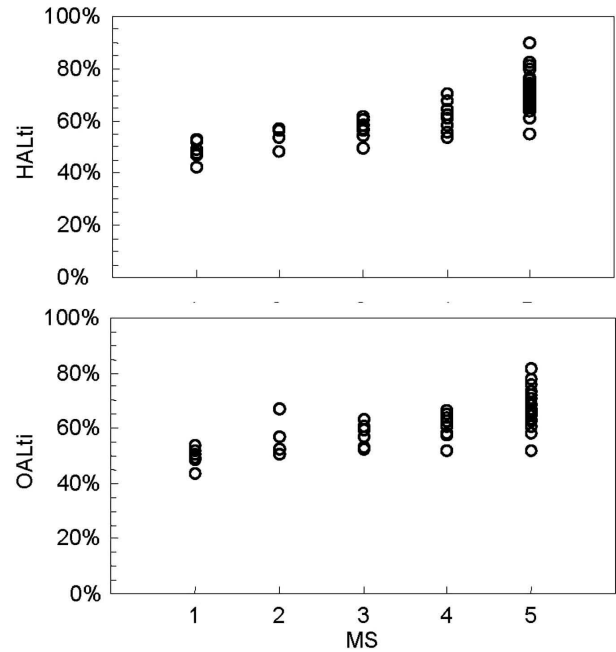


Fig. 4. – Plots of HALti and OALti vs maturity stage (MS)

was not different from 1:1 (50.9%), the percentage of animals with a DL>0 increased with maturity, reaching a maximum of 78.6% at MS=5. The DL ranged from -5.6% to +18.6% (Table 2). The average DL was +1.24%. The DL was about 5%, as reported by Lu (1973), only in fully mature specimens. The average length of the opposite arm was actually greater than that of the hectocotylus, except in fully mature animals. The DL was independent of which arm was modified. Nineteen of the 37 right-armed specimens had a positive DL. In left-armed specimens, this condition was found in 11 of the 23 individuals. The longer ventral arm was also usually more robust.

The hectocotylized arm length (HALt) exhibited a close linear relationship with ML (Fig. 3, top). A very close correlation between opposite arm length (OALt) and ML was also found (Fig. 3, bottom). The averages of HALti were not significantly different among the first three maturity stages. However, HALti increased significantly from MS=3 to 5 (Fig. 4, top). A similar pattern occurred in the opposite arm (Fig. 4, bottom), but in this case the differences among means were significant from MS=3 onward. The average percentages of HALt for the regions of the hectocotylized arm were: HAb=11.4%, HAp=27.7%, HAm=37.4%, and HAd=24.7%. And considering only the fully mature specimens: HAb=10.5%, HAp=28.5%, HAm=34.6%, and HAd=26.3% of HALt.

Development of hectocotylized-arm regions

Hectocotylized Arm, Basal (HAb). Because the HAb has neither suckers nor trabeculae, changes with maturation only involve its length relative to the rest of

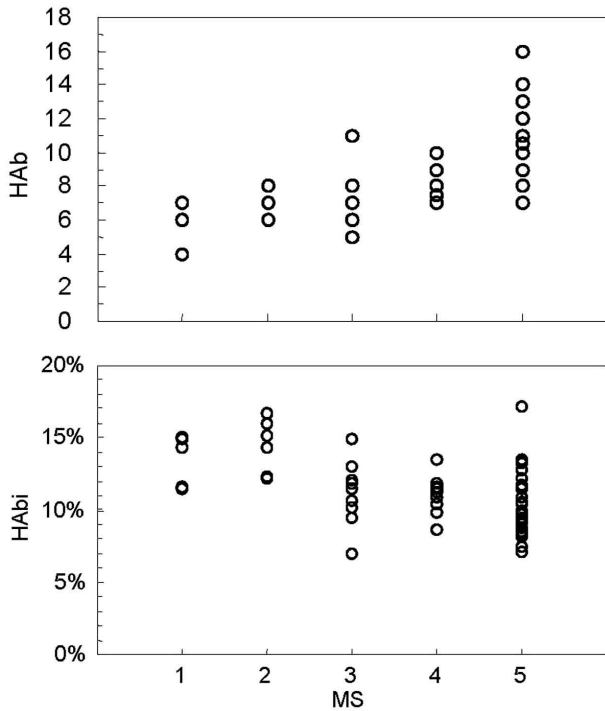


FIG. 5. – Plots of HAB and HAbi vs maturity stage (MS).

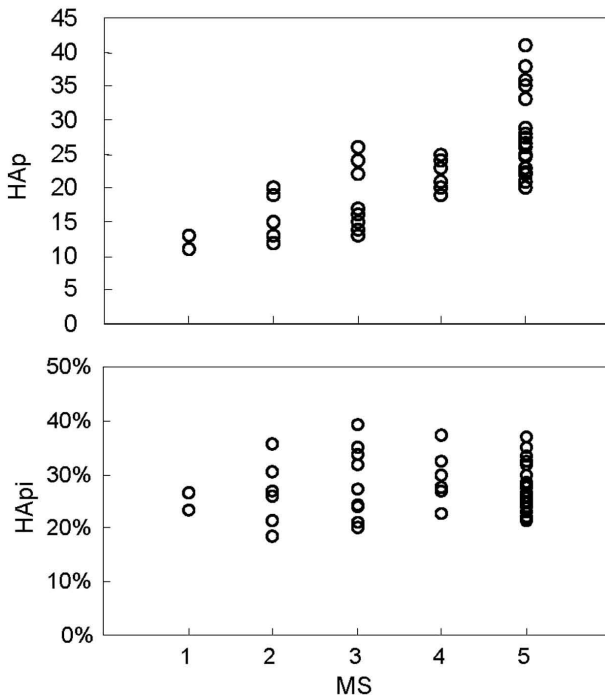


FIG. 6. – Plots of HAp and HApi vs maturity stage (MS).

the arm and to the ML of the squid, as well as growth in absolute length. We found that the length of the Hab varied greatly, ranging from 4 to 16 mm. In fully mature specimens the Hab length ranges from 7 to 16 mm. HAB increases with MS (Fig. 5, top), but the increase in HAbavg is only significant between MS=4 and 5. Al-

though the correlation of HAB with ML is significant, the slope of the regression line is quite low, indicating relatively little change with growth in ML.

HAbi ranges from 7.04% to 17.20% (Fig. 5, bottom), with an average of $11.35 \pm 2.42\%$. The first two maturity stages have similar mean HAbi (ca. 13 to 14%). The index then decreases to a lower, but approximately constant percentage at MS=3 to 5 (ca. 11%).

Hectocotylized Arm, Proximal (Hap). The HAP increases in length linearly with MS (Fig. 6, top). Beginning with MS=4, the differences in HApavg among maturity stages are statistically significant, although the variance tends to increase with the maturity stage. The index HApi is also quite variable (Fig. 6, bottom). The average index changes little among maturity stages. The HApavg in fully mature specimens is $28.46 \pm 4.48\%$.

Hectocotylized Arm, Medial (Ham). Number of suckers and their dentition are addressed below in the section on sucker modification. The dimensions of the flaps at the base of each sucker stalk decrease towards the distal tip of the arm, and the flaps are sometimes absent on the last one or two pairs of suckers on HAM. The flaps are semicircular with a fringed edge that is sometimes invisible without magnification.

A distinct medial part of the arm is first apparent at MS=2 (Fig. 7, top). Only in one specimen was HAM obvious at MS=1. HAM increases with the maturity stage. Although the mean lengths of HAM at MS=2 and MS=3 are not significantly different, differences in length are statistically significant among all other MS segments; HAM grows significantly from MS=3 to 5.

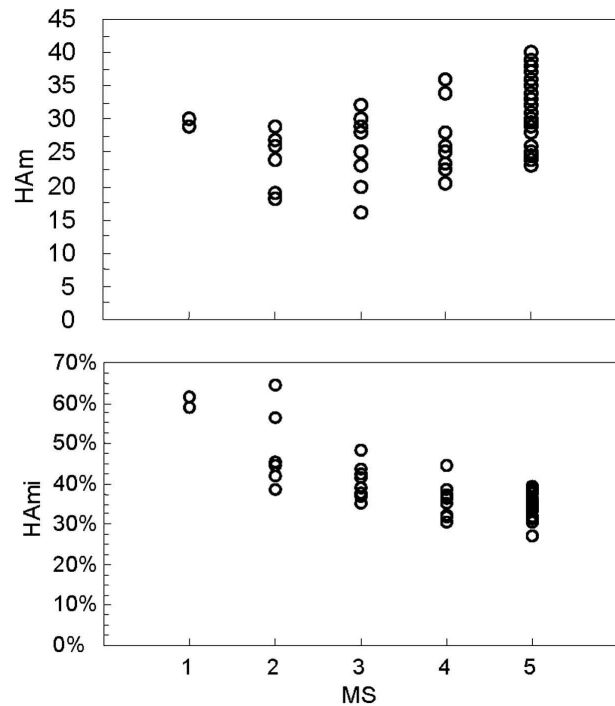


FIG. 7. – Plots of HAm and HAmi vs maturity stage (MS).

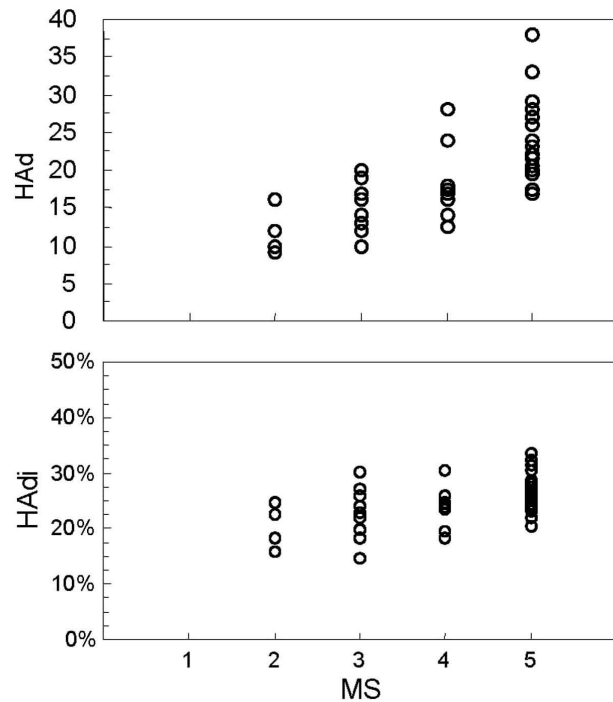


Fig. 8. – Plots of HAd and HAdi vs maturity stage (MS).

The length of the medial part of the hectocotylus also shows a clear linear pattern with ML. The correlation coefficient is one of the highest found in this study. The index of the medial part to the hectocotylized arm length, HAmi, decreases with increasing maturity stages (Fig. 7, bottom). Based on the Tukey-Kramer analysis, this decrease in HAmiavg is significant from MS=2 to 3. Beyond MS=3, HAmi remains essentially constant.

Hectocotylized Arm, Distal (Had). The knobs, papillae and lamellae on the HAd form from sucker stalks. Each sucker cup appears to be resorbed somewhat, then its stalk becomes very weak until the rest of the sucker cup falls off, leaving scars that are sometimes visible on the modified stalk.

Lamellae normally begin to appear in the proximal area of HAd at MS=2. However, in one specimen only, 13 lamellae were present at MS=1. Papillae are present only from MS=3 onward. Therefore, the numbers of lamellae and papillae are unequal throughout the different maturity stages, and sometimes the numbers remain very different even at full maturity when papillae and lamellae are completely formed.

Papillae are usually very indistinct in specimens from the Tyrrhenian Sea, and some are so undeveloped that they are barely visible even with a dissecting microscope; occasionally they are completely absent. The average number of papillae increases significantly when animals reach full maturity. The number of papillae shows a closer relationship with ML ($R=0.480$) than with HALt ($R=0.355$).

In the lamellar row, one or two knobs are present proximal to the real lamellae; occasionally, although

rarely, three or even four knobs occur. Conversely, knobs are sometimes absent. The knobs are conical in form, resembling large, high papillae. The number of knobs does not appear to be related to any of the other characters examined. The number of lamellae varies greatly. The LCAvg generally increases with MS, but this increase is only significant when animals reach full maturity. LC correlates more closely with HALt ($R=0.521$) than with ML ($R=0.496$).

Suckers are absent on HAd except for some minute suckers (diameters about 50-100 μm) with unique dentition at the distal tip of the arm. These minute suckers are frequently found at maturity stage 4 (67% of specimens); however, they were only present in one of the specimens at MS=5 (4%).

HAd lengthens with MS (Fig. 8, top), as does HAdavg. HAd also lengthens with ML. Again, the standard deviation of the y-axis intercept is high, while the linear approximation is quite good, as indicated by the low standard deviation of the ML coefficient.

The taxonomically important index of the length of the distal part to the length of the hectocotylized arm, HAdi, increases with MS (Fig. 8, bottom). HAdiavg increases significantly with each subsequent maturity stage. Although HAdiavg is about 25% when all MS are included, it is >26% for fully mature squid.

Suckers

Suckers are present on HAp and HAm. The suckers on HAp are similar in size to the corresponding suckers on the opposite arm. Their diameter on fully mature specimens ranges from 1.3 to 2.4 mm, with an average of 1.8 ± 0.3 mm. SDpavg increases with MS, and this increase is statistically significant from maturity stage 2 onward to maturity. The diameter of these proximal suckers is more closely correlated with HALt than with ML. The SDpi remains approximately constant at about 2% in the final three maturity stages. The differences are not statistically significant at the $P=0.95$ level.

The sucker diameter on HAm, SDm, ranges in adult specimens from 0.3 to 0.8 mm, with the exception of one adult that had extremely small suckers of 0.08 mm in diameter. At MS=5, the SDmavg is 0.6 ± 0.2 mm. This distinct difference in size from more proximally located suckers is not found on the opposite arm, where the sucker size gradually diminishes distally.

SDm increases significantly from MS=2 onward. This parameter is more closely correlated with HALt than with ML. The SDmi also increases from MS=2 onward, although the increase from MS=4 to MS=5 is not statistically significant.

The number of the suckers (SC) on the different parts of the hectocotylized arm does not vary with maturity stage, mantle length or hectocotylized arm length. The SC on HAp ranges from 10 to 16 in adult specimens. The mean SC on HAp of the fully mature specimens is 11.96 ± 1.45 suckers; the mode is 12. The range of SC on HAm is 12 to 30, although in fully ma-

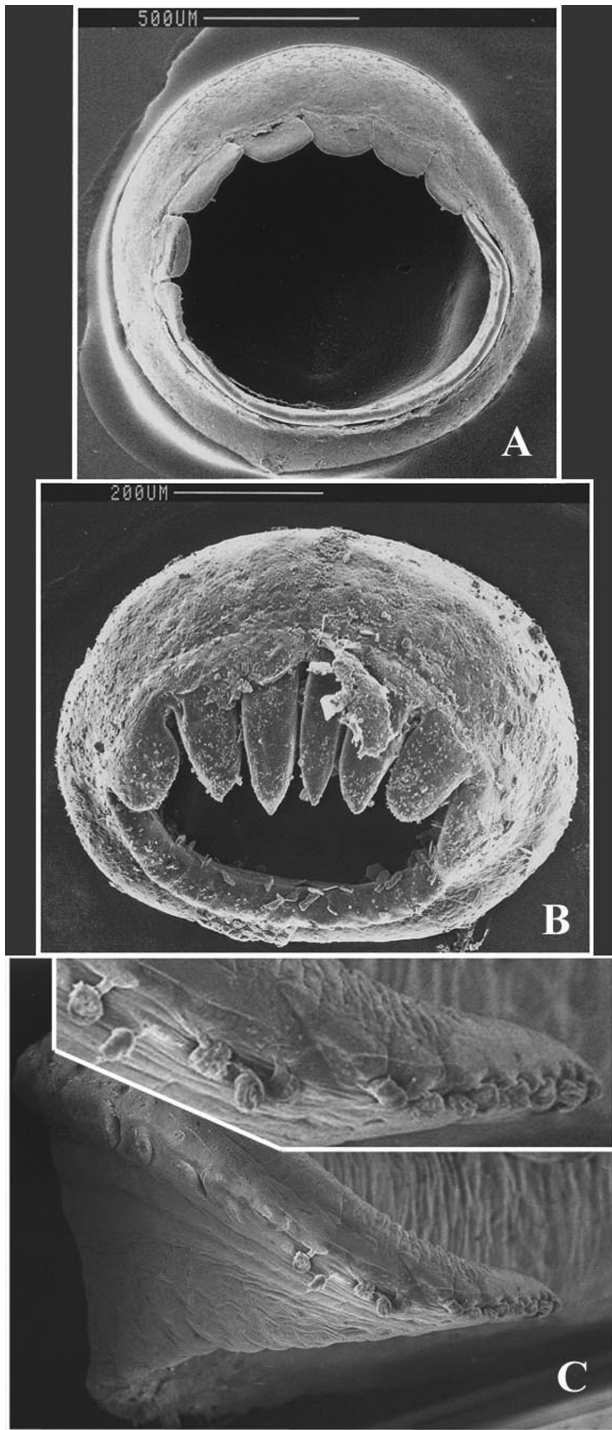


FIG. 9. – Scanning electron micrographs of A) dentition of proximal (4th) sucker; B) dentition of medial (17th) sucker; C) distal region of hectocotylus, with inset closeup of minute suckers (50 to 200 μm diameter).

ture specimens, the mean SC on HAm is 23.29 ± 3.62 , and the mode is 24 suckers.

Fourteen suckers on the HAp of the adult animal were selected for S.E.M. study. The 11 proximal suckers have essentially identical dentition, with 7 to 9 low, rounded teeth (Fig. 9A). Distally, beginning with the

12th sucker, the dentition begins to resemble that on the suckers of the HAm, with pointed teeth that are longer in the center than to the sides. The 15th sucker is the first of the HAm and all teeth are long and pointed. Distally from there, the dentition on HAm suckers remains approximately the same, with 7 to 9 long, pointed teeth (Fig. 9B). The central teeth are usually somewhat longer than the lateral ones.

Sucker dentition differs along the length of the arm on both hectocotylyzed and opposite arms, in juveniles as well as adults. The dentition already differs between suckers of HAp and HAm at MS=1 or 2; instead of the gradual change of shape in sucker dentition visible in juveniles from the proximal to distal arm segments, an abrupt change occurs in the last two or three suckers of the HAp. The dentition of most suckers on the HAp consists of a chitinous ring with 9 to 11 comparatively short, rounded teeth. The dentition on HAm is different, and actually includes the last one or two suckers on the HAp, with 7 to 9 teeth that are longer and more pointed than those of the HAp. The last lateral tooth on each side is rounded, while the others are very pointed and elongate. Intermediate forms of dentition occur in the border zone between the HAp and HAm. The number of pointed teeth increases from the proximal to the distal suckers on the HAm. Minute suckers (diameter 50 mm to 200 mm) occur at the tips of both hectocotylyzed and opposite arms with a different pattern of dentition of 10 to 14 miniscule pointed teeth. The teeth are not limited to the outer rim, but occur all around the inner surface of the cylinder that forms the chitinous ring, just below its upper border.

The diameter of the suckers on the HAp increases with MS as well as with ML and HALt, showing an asymptotic pattern that reaches a maximum diameter at full maturity. The size of the suckers on HAp increases until the 4th row of suckers, then decreases distally, whereas the diameter of the suckers on the HAm decreases consistently towards the tip of the arm.

Spermatophores

The average number of spermatophores in our specimens was 236.4 ± 67.5 per squid at MS=5, with a broad range of 152 to 379. The average length of spermatophores was significantly correlated with ML, HALt, HAd and HAm (Table 1). No obvious pattern was detected for any other variables, and the correlation coefficient was not statistically significant.

DISCUSSION

Growth of the hectocotylyzed arm

Each of the component sections of the hectocotylyzed arm grows along its entire length rather than growing simply at either the distal or proximal end. All regions grow with the increase in both the MS and ML. Conversely, the number of suckers stays essen-

tially constant on the HAp and HAm, and the suckers are always evenly spaced. The growth rate of the regions of the hectocotylized arm in relation to the mantle length can be represented best by indices. HAbi stays approximately constant until the animal reaches MS=3, then decreases from MS=3 to MS=5. Meanwhile, HApi increases very slowly with MS, staying almost constant, and HAmi decreases quickly with MS until MS=3, when HAd forms. After that, the decrease in HAmi is no longer significant. Relative growth of HAm and HAb slows while HAd grows quickly. Contrary to the observations of Coelho *et al.* (1985), the index of the HALt to ML (HALti) exhibits a well-defined curve pattern that increases its slope with increasing MS. Thus, the hectocotylized arm grows faster as the animal approaches maturity. The pattern is similar for the opposite arm, but the index of the length of the hectocotylized arm, HALti, is generally larger in fully mature squid than the corresponding index of the opposite arm, OALti.

The length of the hectocotylized arm is usually only greater than that of the opposite arm in adult specimens (MS=5), but this difference is not entirely consistent. In about 22% of fully mature animals the opposite arm is longer. Whereas Lu (1973) and Coelho *et al.* (1985) stated that sometimes the hectocotylized arm is neither longer nor more robust than the opposite arm, we found this inconsistency to be true only at full maturity. At full maturity, most of these squid possessed a hectocotylized arm that was about 5.3% longer than the opposite arm, but with variability (SD= 6.6%) greater than the mean difference in length.

Most authors (e.g. Lu 1973, Nesis 1987, Roper *et al.* 1998) use the index of HAd to HALt (i.e., HAdi in this study) as a key character for identifying *Illex* species. The average of HAdi found in the present work over all maturity stages (HAdiavg=24.7%) matches that reported by other authors (25%). In fully mature specimens, HAdi is >26%, approaching that of *I. oxygonius* (29%). Therefore, MS should be considered when HAdi and other indices are used for identification. Although we assessed the details of hectocotylus growth in a geographically limited population, these results indicate that caution is warranted when hectocotylus characters are used for identification of *Illex* species.

Spermatophore length is related to the total length of the hectocotylized arm, and to the component lengths of both the medial part and tip of the hectocotylized arm, as well as the mantle length. These relationships may reflect a functional necessity at full maturity; a longer tip is probably necessary and more efficient for manipulating and implanting longer spermatophores.

Sucker modification and dentition

Zuev (1966) reported suckers on the hectocotylized arm tips of adult *I. coindetii* from the Mediterranean Sea, but Aldrich and Lu (1968) questioned the presence of suckers on the tip of the hectocotyl-

ized arm of *I. illecebrosus* from the western North Atlantic. Based on our study, there are usually no minute suckers on the tip of the hectocotylus of fully mature Mediterranean specimens of *I. coindetii*. Suckers are added distally on the arm as the animal grows. The tips of both hectocotylized and opposite arms have minute suckers (diameter 50 to 200 μm) with a distinctive dentition pattern. These minute terminal suckers are absent on the distal tip of the hectocotylus in fully mature specimens, whereas they are usually present in early mature specimens. On juveniles, another unusual type of sucker, with the chitinous ring covered by a fleshy layer, is found near the base of the arm at the border between HAb and HAp. However, the dentition is similar to that of HAp suckers.

Fringed flaps, lamellae and papillae

Flaps and modified dentition clearly distinguish HAm from HAp. Beginning with MS=3, flaps form from the trabeculae present along the arm. The proximal-most flaps correspond to the first suckers that have fully modified dentition on HAm. Flap formation is not concurrent with the formation of lamellae. Sometimes the flaps form prior to the formation of papillae, as some animals have flaps but no papillae, whereas the reverse situation has not been found.

Formation of papillae and lamellae seems quite rapid. The distal suckers (except for the minute arm-tip suckers) appear to be modified and then shed, leaving the bases on HAd to transform into lamellae and papillae. Lamellae form first but papillae probably form more rapidly as they are more pointed and elongate (i.e., less modified from the shape of the original sucker stalks). Papillae begin to form at MS=3, whereas the more-modified lamellae begin at MS=2.

Development of the hectocotylus

A priori we proposed that the modifications that comprise the hectocotylized arm could develop in four possible ways:

1. Modifications form proximally to distally on the arm. HAm would form before HAd. The first suckers that transform into knobs, lamellae and papillae would be the ones closest to the border of HAm and HAd, and the last ones to form would be those at the distal end of the hectocotylus. Knobs would be formed before lamellae.

2. Modifications form distally to proximally. The first suckers lost, with stalks subsequently modified, would be those on the distal end of the arm, whereas the last suckers to be lost would be proximally on the border of HAm and HAd. Knobs on the lamellae row would be the last modification to appear.

3. The modifications originate from a medial point on the arm, perhaps at the border of HAm and HAd, and proceed in both directions, distally and proximally.

4. All modifications occur simultaneously, so that the hectocotylus develops as a single unit.

Our observations are not at all consistent with hypotheses 2 or 3. None of the parameters we examined was consistent with modifications forming distally to proximally along the arm, nor originating at some medial point and dispersing both proximally and distally. Minor indications of progression from proximal to distal were observed. In reality, the first indication of modification is the reduction in relative sucker size and an alteration in sucker dentition on HAM at MS=1 or 2. Furthermore, suckers are added distally as the arm grows. Suckers at the arm tip are progressively transformed into lamellae and papillae on HAD until full maturity is reached. However, the general pattern that we observed in the development of the hectocotylus was that the various modifications essentially occur simultaneously at about MS=2 to 3. Flaps begin to form on HAM at MS=3. Lamellae begin to form on HAD at MS=2 and papillae at MS=3. Therefore, we believe that the best description for hectocotylus development in *I. coindetii* is intermediate between hypotheses 1 and 4. That is, modifications (e.g., sucker size/dentition, flaps, lamellae, papillae) essentially occur simultaneously, but the distal arm tip continues to grow slightly.

Relationships with other species of the genus *Illex*

Although *Illex coindetii* has the most extensively modified hectocotylus in the genus, it does not pass through an *illecebrosus*-like stage; suckers on HAM are smaller than those on HAP as soon as hectocotyliation begins. In addition, we have never seen an *I. coindetii* hectocotylus with lamellae and papillae on the distal tip that did not also have fringed flaps on HAM. Thus, the differences between *I. coindetii* and *I. illecebrosus* are recognizable as soon as the hectocotylus begins to form, even though the HADi is similar to that of *I. illecebrosus* (22%) during MS=2 (the HADi is about 22% in *I. coindetii*) and MS=4 (the HADi is about 23% in *I. coindetii*). Since the hectocotylus of *I. oxygonius* has an index of the distal tip length (our HADi) that reaches 29% of HALt, and has no HAM region, it cannot be confused with *I. coindetii* either. *Illex argentinus* is a geographically separated species with a very distinctive hectocotylus, and thus taxonomic confusion is not a problem for this species.

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APPENDIX 1. Definitions of maturity stages used in this study.

- 1 = IMMATURE. Sexual organs are not visible (spermatophoric complex may be barely distinguishable).
 2 = PRECURSORY. Needham's sac is whitish; all internal sexual organs are distinguishable.
 3 = MATURING. Needham's sac and the sexual organs are completely developed; immature spermatophores are sometimes present.
 4 = EARLY MATURE. First few fully developed spermatophores occur in the still flaccid Needham's sac.
 5 = FULLY MATURE. Needham's sac is turgid and fully packed with numerous spermatophores.
 6 = SPENT. Very few spermatophores, usually burst, remain in Needham's sac; the testis is degenerating. (This stage is not considered in the present work because the structure of the hectocotylus is unchanged from that of the fully mature stage).

APPENDIX 2. Abbreviations used in the text. Measurements are in mm.

- ML: Dorsal mantle length.
 MS: Maturity stage.
 HALt: Total length of hectocotylized arm from proximal (basal) V-notch to distal tip; HALt=HAb + Hap + Ham + HAd (see Fig. 1).
 HALti: Index of HALt as percentage of ML.
 OALt: Total length of the opposite arm from proximal (basal) V-notch to distal tip.
 OALti: Index of OALt as a percentage of ML.
 HAb: Basal region of the hectocotylized arm without suckers, from V-notch to the first sucker; also used to indicate length of basal region.
 HAbi: Index of HAb as a percentage of HALt.
 HAp: Proximal region of hectocotylized arm bearing normal-sized suckers; also used to indicate length of proximal region.
 HApi: Index of HAp as a percentage of HALt.
 HAm: Medial region of hectocotylized arm bearing reduced-diameter suckers; also used to indicate length of medial region.
 HAmi: Index of HAm as a percentage of HALt.
 HAd: Distal region (tip) of hectocotylized arm bearing papillae and lamellae in place of suckers; also used to indicate length of distal region.
 HAdi: Index of HAd as a percentage of HALt.
 SDp: Greatest diameter of the chitinous ring visible on normal suckers on HAp.
 SDpi: Index of the greatest diameter of the chitinous ring visible on normal suckers on HAp as a percentage of ML.
 SDm: Greatest diameter of the chitinous ring visible on small suckers on HAm.
 SDmi: Index of the greatest diameter of the chitinous ring visible on the smallest suckers on HAm as a percentage of ML.
 SC: Number of suckers on a given region of the arm (e.g., SC on HAm).
 LC: Number of lamellae on HAd; this includes the number of knobs present on the lamellae row, unless otherwise noted.
 PC: Number of papillae on HAd.
 DL: Difference in length, as a percentage, between the hectocotylized arm and opposite arm; $DL=100[(HALt - OALt)/OALt]$.
 SpL: Average length of measured spermatophores.
 XXXmin: Minimum measure or index XXX.
 XXXavg: Average measure or index XXX.
 XXXmax: Maximum measure or index XXX.
 R: correlation coefficient of variables in the linear regression.
 df: degree of freedom for statistical test