A phylogenetic study of the squid family Onychoteuthidae (Cephalopoda: Oegopsida)

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The oegopsid squid family Onychoteuthidae presently comprises six genera (*Moroteuthis*, *Onychoteuthis*, *Ancistroteuthis*, *Kondakovia*, *Onykia* and *Chaunoteuthis*) but the status of some of these is still uncertain. An interdisciplinary study was undertaken to clarify the phylogenetic relationships, which included a morphological approach (morphometric analysis), and a molecular study of a mitochondrial gene portion (1-rRNA gene, 16S).

The morphometric analysis identified two groups, one including some *Onychoteuthis* and some *Moroteuthis knipovitchi*, and another including the remaining genera or species. At the intrageneric level, *M. knipovitchi* appears to be separated from the other species of the genus; *M. robusta* is closely related to *M. ingens* and *M. pacifica*. Morphometric analysis confirmed that *Kondakovia longimana* is different from *M. ingens*. Likewise, *Onychoteuthis* was clearly separated from the other genera, but there is neither geographical grouping nor morphometric differentiation of the *Onychoteuthis* species with the parameters measured. Some specimens were apparently intermediate between the *Onychoteuthis* and *Ancistroteuthis* groups.

On a molecular basis, the Onychoteuthidae appeared to be monophyletic. Monophyly of the genus *Moroteuthis* (four species studied) was not strongly supported: *M. knipovitchi* was distinct from the others. The molecular analysis showed three Hawaiian species, *Onychoteuthis compacta*, *O. sp. B* and *O. sp. C*, to be closely related. The gene sequence for the newly created species of *Onykia* is clearly different from all the others, indicating that it is a true species.

Keywords: phylogeny; oegopsid squids; morphometry; mt-l-rDNA sequences

1. INTRODUCTION

Studies of systematics and phylogenetics increasingly include molecular as well as morphological data. When both methods agree, this dual approach can be powerful. In cephalopods, and especially in oegopsid squids, many difficult systematic problems require such an approach. The oegopsid squids (order Teuthoidea) are comprised of 23 families, many of which have uncertain or unknown relationships among genera. The Onychoteuthidae is such a family. This family is presently considered to contain six genera and 13 species (table 1), all possessing two rows of hooks on the manus of the tentacular club (no hooks on arms). Many are extremely similar in appearance; the main generic diagnostic characters are illustrated in figure 1. Some of the species are thought to be cosmopolitan, whereas others have a regional distribution and the present systematics of the family are unsatisfactory. Undescribed species may exist, yet the separation of known species is often difficult and the relationships among species and genera are uncertain. A recent study on cephalopod phylogeny using molecular methods has provided evidence of the utility of such an approach to resolve the uncertain relationships of some decapod families (oegopsid and myopsid squids)

(Bonnaud *et al.* 1994, 1997). An interdisciplinary study of the Onychoteuthidae thus appeared feasible. The present study especially focuses on relationships among Southern Ocean onychoteuthids, an important resource for vertebrate predators in these waters. It forms part of ongoing studies on trophic relationships of cephalopods in this region (Rodhouse 1989; Rodhouse & Prince 1993). The morphological study is based on conventional morphometrics and the molecular study is based on the analysis of a mitochondrial gene portion (1-rRNA gene, 16S).

2. MATERIAL AND METHODS

(a) Morphological study

The morphological study is based on morphometrics, which allows an objective methodology (see below) that can be uniformly applied across all species and genera. Other morphological characters that are useful in separating species and genera (e.g. club suckers' arrangement, skin ornamentation, presence and shape of photophores, shape of hooks, mantle and tentacle pigmentation) are not readily applicable to this type of analysis and have not been included.

The number of specimens studied per group (genus or species) varied: some are well represented, others are represented by a single individual (*ca.* 20 *Moroteuthis ingens*, one *M. robsoni* and one

Table 1. *Genera and species in the family Onychoteuthidae (from Nesis 1987)*

Family Onychoteuthidae Gray, 1849
Genus <i>Moroteuthis</i> Verrill, 1881
<i>M. ingens</i> (Smith 1881)
<i>M. robsoni</i> Adam, 1962
<i>M. robusta</i> (<i>M. pacifica</i> Okutani, 1983) (Verrill, 1876)
<i>M. lönnbergi</i> Ishikawa & Wakiya, 1914
<i>M. knipovitchi</i> Filippova, 1972
<i>M. aequatorialis</i> Thiele, 1920
Genus <i>Onychoteuthis</i> Lichtenstein, 1818
<i>O. banksii</i> (Leach, 1817)
<i>O. borealijaponicus</i> Okada, 1927
<i>O. compacta</i> (Berry, 1913)
<i>O. meridiopacifica</i> Rancurel & Okutani, 1990
Genus <i>Onykia</i> Lesueur, 1821
<i>O. carriboea</i> Lesueur, 1821 (= <i>O. rancureli</i> Okutani, 1981)
Genus <i>Ancistroteuthis</i> Gray, 1849
<i>A. lichtensteini</i> (Férussac 1835 in Férussac & d'Orbigny, 1834–1848)
Genus <i>Kondakovia</i> Filippova, 1972
<i>K. longimana</i> Filippova, 1972
Genus <i>Chaunoteuthis</i> Appellöf, 1890
<i>C. mollis</i> Appellöf, 1891

M. robusta). The characteristics and origin of each sample are given in table A1, Appendix A.

A total of 150 specimens was measured according to the recommendations of Roper & Voss (1983), using 0.10 mm Vernier callipers for the smaller specimens and a graduated ruler for the larger ones. The following measurements were taken: mantle length (ML), fin length and width (FL and FW), lengths of all right arms (AL) and right tentacle length (TL), tentacular club length (CL) and carpus length (LL). Head width and mantle width, which can undergo important variations during fixation, were not considered. Other possible measurements were abandoned (dactylus length, lower beak length) because the animals were either too small, or the structure (beak) was difficult to examine without damaging the specimen. Incomplete specimens or those in bad condition (no right tentacle, torn fin, etc.) were discarded. The characters needed to be present and measurable in all specimens because the statistical package used could not analyse files with missing values.

Most of the specimens had been formalin-fixed and then preserved in 70% ethanol. Some had been fixed and were still preserved in 'spirit'. We do not know, *a priori*, the impact of such treatment on the organs' total and relative lengths. A small number of specimens, including two *Moroteuthis ingens* and one group called 'group Y' identified (by C. C. Lu) as *Kondakovia longimana*, were frozen before being formalin-fixed.

The monospecific genus *Chaunoteuthis* (*Chaunoteuthis mollis*, Appellöf, 1891) was not taken into account in this study. The type was not available, and the taxon might not be a valid one (Kubodera *et al.* 1998). Recently, Arkhipkin & Nigmatullin (1997) identified *C. mollis* as the mature female of *Onychoteuthis banksii*.

Some meristic characters were considered in all specimens: the number of suckers on right arm II, the number of suckers

and hooks on the club's manus, the number of suckers and pads on the club's carpus and the number of suckers (approximately) on the dactylus. However, they were not variable enough among the genera to allow discrimination.

All data for the ten measurements on each specimen were log-transformed (ln) and analysed by principal components analysis (PCA) using a variance–covariance matrix (giving the largest weights to the largest variances) with NTSYS computer software (Rohlf 1990).

(b) *Molecular study*

Specimens from existing collections were mostly fixed in formalin, which is unsuitable for molecular analyses. DNA extraction requires frozen or ethanol-preserved material, which is rare in collections of oceanic species. Frozen or ethanol-preserved tissues were available from *Onykia* sp. (a form similar to *O. rancureli*), *Onychoteuthis compacta*, *Onychoteuthis* sp. B and *Onychoteuthis* sp. C (paralarvae identified by Young & Harman (1987)), *Onychoteuthis* sp. (undescribed new species), *Moroteuthis robsoni*, *M. robusta*, *M. knipovitchi* and from many specimens of *M. ingens* (Appendix B). The gene portion chosen for sequencing was the 3' extremity of the gene coding for the mitochondrial 16S RNA. This gene has proved to be useful for phylogenetic analyses at the infrafamilial level in decapod cephalopods (Bonnaud *et al.* 1994).

Total DNA was obtained following a CTAB method extraction: a small piece of tissue was incubated at 60 °C for between 30 min and 1 h in a CTAB buffer (Tris 100 mM pH 8, EDTA 20 mM, pH 8, NaCl 1.4 M, CTAB 2%, β -mercaptoethanol 0.2%) with 0.4 mg ml⁻¹ of proteinase K and 1% SDS. Chloroform isoamyl alcohol extractions were repeated twice, followed by DNA precipitation in ethanol and sodium acetate (0.3 M final concentration). Amplifications were performed using two primers, 16Sar and 16Sbr, according to the classification established by Simon *et al.* (1991). After cloning in P-Mos blue vector (Amersham), sequencing (one strand) was performed by the dideoxy chain-termination method, with the primers located in the plasmid sequence (EMBL accession numbers in table B1, Appendix B). The sequences were aligned by eye. They were analysed, taking all differences into account, by the neighbour-joining distance method, available in the MUST package (Philippe 1992), and by the PAUP 3.1 parsimony method (Swofford 1985), using heuristic and branch-and-bound treatments. Bootstrap resampling procedure was performed in both cases to measure the support for each of the clades on the phylogenetic trees.

3. RESULTS AND DISCUSSION

(a) *Morphological study*

Figures 2, 3 and 5 illustrate the results of the statistical analysis based on morphological measurements. Only axis 1 and axis 2 were considered (table 2): they explain 79.8% of the variance observed (70.4% for axis 1). Fin dimensions, especially fin length (FL), are particularly important on axis 1, whereas total length (TL) is an important factor on axis 2.

Figure 2 relates axis 1 to size axis, i.e. the mean value of log-transformed measures of the ten length variables (ML, AL1 to AL4, FL, FW, TL, CL and LL). It shows that there does not appear to be any substantial change in allometric relations between young and adult specimens over the size range measured. Each cluster, corresponding to one

Figure 1. Main generic diagnostic characters within the family Onychoteuthidae.

Table 2. Factor weights for principal components analysis axis 1 (PC1) and axis 2 (PC2) on each variable

variable	PC1	PC2
ML	0.396	-0.047
FL	0.544	0.465
FW	0.421	-0.325
AL1	-0.296	0.009
AL2	-0.319	-0.071
AL3	-0.213	-0.005
AL4	-0.253	-0.007
TL	-0.251	0.621
CL	0.049	-0.117
LL	-0.078	-0.522

species or to a group of identical specimens, is parallel to the size axis: this means that variation due to growth cannot be recognized over general variation. The analysis of all genera (figure 3) isolates one group, including some *Onychoteuthis* and some *Moroteuthis knipovitchi*, with positive scores on axis 1, and the other genera or species with negative axis 1 scores. The morphometric analysis separates one species (*M. knipovitchi*) from the other species of the genus but is not discriminative enough to distinguish a typical conformation corresponding to the other *Moroteuthis* species. Likewise, the genus *Onychoteuthis* is clearly separated from the other genera.

When analysing the relationships within genera, more detailed conclusions can be drawn.

(i) Genus *Kondakovia* Filippova, 1972 (figure 3)

This genus is monospecific. *Kondakovia longimana* is an Antarctic species that differs from *M. ingens* by the

presence of a double row of suckers in addition to hooks on the manus in adults. The adult specimen studied displays few morphological differences from *M. ingens*, as Kubodera *et al.* (1998) have observed. On the contrary, PCA shows that young specimens of *K. longimana* display marked morphometric differences as regards juvenile *M. ingens*. Two groups of young *Kondakovia* can be recognized from PCA, which confirms the general observations made during the measurements on some specimens (general aspect, dactylus form and tentacle length). One of the *Kondakovia* groups is close to specimens of young *M. ingens*. Many young *M. ingens* have a double row of suckers on the club's manus, a typical *K. longimana* character, as reported by Tsuchiya & Okutani (1991). On the basis of our morphometric results, however, this genus seems different from *M. ingens*.

Group Y. Approximately ten frozen specimens from the shelf around the Falkland Islands have morphological characters of juvenile *K. longimana*, including suckers on the club's manus, but their nuchal folds are difficult to distinguish (when present). They were identified by C. C. Lu as probable young *K. longimana*. The morphometric analysis shows that they do not cluster with the other *K. longimana*, but this may be because of the poor condition of the frozen specimens. However, a scanning electron microscope (SEM) analysis of the radula of the adult specimen and of these juveniles also shows a different structure (figure 4). In particular, the lateral cusps are distinctly more developed on the rhachidian teeth of the group Y specimen.

(ii) Genus *Moroteuthis* Verrill, 1881 (figure 5)

Seven species of the genus, including adults and young, were examined. The specimens of *M. knipovitchi* were very distinct from the other adult *Moroteuthis*. One of the

Figure 2. Axis 1 and size axis (mean value of log-transformed length variables) after principal components analysis of ten variables. Key to symbols: open triangles, *Moroteuthis* sp.; filled triangles, *Kondakovia longimana*; open circles, *Onychoteuthis* sp.; filled circles, *Ancistroteuthis lichtensteini*; 'plus' signs, *Onykia* sp.; squares, group Y.

Figure 3. Axis 1 and axis 2 after principal components analysis. Symbols are identical to those in figure 2. On this figure, two groups of juvenile *Kondakovia longimana* are indicated.

specimens of *Moroteuthis lönnbergi* and the specimen of *M. robsoni* also appear to be separated from a group that includes *M. aequatorialis*, *M. robusta*, *M. pacifica* and *M. ingens*. The two specimens of *M. lönnbergi* were very distant from each other according to the morphometric analysis, although they come from the same area, were captured the same day, and appear to be at the same stage of sexual maturity. As far as *M. aequatorialis* and *M. pacifica* are concerned, their grouping confirms what Kubodera *et al.* (1998) suggest: these two species might be

morphological variants of *M. ingens*. Nesis (1987) considers *M. pacifica* to be a synonym of *M. robusta*: our analysis shows a grouping of these two species with other specimens of *M. ingens*. Some *Moroteuthis*, assigned to *M. ingens*, appeared to be different from the others in their colour and general appearance (not due to conditions of fixation). The morphometric analysis groups them with the majority of the other *M. ingens*, and SEM analysis of their radula shows that it is identical to that of other *M. ingens*

Figure 4. Radula of (a) *Kondakovia longimana* (adult) and (b) one specimen of group Y (SEM views).

analysed in parallel. These specimens could be morphological variants despite the fact that they came from the same geographical area.

(iii) *Genus Ancistroteuthis* Gray, 1849 (figure 5)

This genus is monospecific. The species *Ancistroteuthis lichtensteini* is known from the Mediterranean and from the tropical and subtropical Atlantic. The specimens studied here came from the Mediterranean. In spite of an apparently homogeneous grouping of the available specimens, three of them (including a syntype of *Onychoteuthis lichtensteini*) are distinct from the others. Their tentacles and arms are proportionally smaller relative to the rest of the body than those of the other *A. lichtensteini*. PCA locates the data on the right side of axis 2 and they group with some *Onychoteuthis* species. Kubodera *et al.* (1998) split *A. lichtensteini* from the Pacific and Atlantic into four distinct forms, but no Mediterranean specimens were taken into account by these authors. Morphometric comparisons of the Mediterranean specimens with those from other areas would be valuable.

(iv) *Genus Onychoteuthis* Lichtenstein, 1818 (figure 5)

This genus is said to include at least four species (table 1). They are distinguished on the basis of the photophores and the number of hooks on the manus of the tentacular club. One species, *Onychoteuthis bartlingii* Lesueur 1821, was already synonymized with *O. banksii* by Férussac &

d'Orbigny (1834–1848). The two *O. borealijaponicus* included here cannot be clearly distinguished from the *O. banksii*. The photophores of the *O. banksii* from Japan are hardly visible. The numbers of hooks are 26 in the two *O. borealijaponicus* examined, and 22, 23, 26 and 27 in the four Japanese *O. banksii*, whereas the numbers of hooks are 20–24 in the *O. banksii* from other areas (*ca.* 25 specimens). The latter are closely related to the Japanese *O. banksii* in terms of morphometrics, but they have very different photophores. There is no geographical grouping. The morphometrics of the specimens appear quite homogeneous. Three of the specimens occupy a noteworthy position in relation to the main group: they appear close to axis 2 and group with the three *Ancistroteuthis lichtensteini* described earlier. Based on morphometric data only, two explanations seem possible: either there is a morphological continuum between *Ancistroteuthis* and *Onychoteuthis*, or there is an intermediate group at the intersection of the two genera, which would constitute a distinct species. Other criteria (*i.e.* gladius and photophores) may support this hypothesis but were not examined in this study.

(v) *Genus Onykia* Lesueur, 1821 (figure 5)

The status of this genus, as separate from *Moroteuthis*, is questionable (Tsuchiya & Okutani 1991). Some of the species, at least, seem to be the young stages of species the presently placed in the genus *Moroteuthis*. One species (*Onykia rancureli*), however, may represent a new genus (Kubodera *et al.* 1998). Four specimens of two putative species were examined: *O. rancureli* Okutani, 1981 and *O. indica* Okutani, 1981. They appear to be two different species. However, it must be noted that the two specimens of *O. indica* are distant from each other and that they are similar in PCA scores to *K. longimana*. The paratype and the holotype of *O. rancureli* are likewise very distant from each other in terms of morphometry.

(b) **Molecular study**

The outgroup chosen consisted of two species of another oegopsid family, the Enoploteuthidae, which share with the Onychoteuthidae two rows of hooks on the manus of the tentacular club. There are no differences between the two nucleotide sequences of *Onychoteuthis* sp. B and *Onychoteuthis* sp. C. The two species have been separated mainly on the basis of paralarval chromatophore patterns. According to molecular results they either belong to the same species, the juveniles of which display polymorphic chromatophore patterns, or the gene portion sequenced is not variable enough to detect nucleotide differences between the two species.

The phylogenetic trees obtained were similar on the basis of both distance and parsimony methods. Figure 6 illustrates the neighbour-joining analysis of the sequences; only bootstrap values higher than 50% are significant. The Onychoteuthidae appear monophyletic on the basis of the species studied and relative to the outgroup. *Onychoteuthis compacta* is very close to *Onychoteuthis* sp. B and *Onychoteuthis* sp. C, but the fourth *Onychoteuthis* sp. is not linked to the others with a strong bootstrap value. R. E. Young (personal communication) has indicated that this specimen is similar to *O. compacta*, but represents a new species of

Figure 5. Relative position of species of *Moroteuthis*, *Onykia*, *Onychoteuthis* and *Ancistroteuthis* on axis 1 and axis 2 after principal components analysis. *Kondakovia* and group Y are identified by a filled triangle. *Moroteuthis*: I, *M. ingens* adult; i, *M. ingens* juvenile; P, *M. pacifica*; Q, *M. aequatorialis*; U, *M. robusta*; R, *M. robsoni*; L, *M. lönnbergi*; K, *M. knipovitchi*. *Onykia*: C, *O. rancureli*; N, *O. indica*. *Onychoteuthis*: filled circle within triangle, *O. bartlingii*; filled circle within square, *O. borealijaponicus*; filled circle, *O. banksii*. *Ancistroteuthis*: filled circle within circle, *A. lichtensteini*.

Figure 6. Tree obtained after neighbour-joining analysis of 16S sequences (all sites taken into account); only bootstrap values higher than 50% are indicated.

Onychoteuthis which is now being described (Seki 1998). We conclude from our data that this new species is not as closely related to *O. compacta*, *Onychoteuthis* sp. B and *Onychoteuthis* sp. C as these species are to each other.

Monophyly of the genus *Moroteuthis* including the four species is not supported. However, *M. robusta*, *M. robsoni* and *M. ingens* appear very close to each other: the node for their grouping is supported by a high bootstrap value (over 90%). This result is congruent with the morphometric analysis as also is the distance between *M. knipovitchi* and these three species.

The specimen of *Onykia* studied is not closely related to any other species: the sequence is clearly different from the others, indicating that it could be a valid genus. More specimens, however, need to be sequenced before reaching a conclusion on the matter. Material suitable for mol-

ecular analysis is necessary to determine the position of *K. longimana*.

4. CONCLUSIONS

There seem to be many morphometric variants of *M. ingens* that do not appear in the nucleotide variations observed, but, unlike the molecular material, the specimens analysed morphologically came from two different geographical areas.

Moroteuthis robusta is closely related to *M. ingens* and *M. pacifica*. *M. robusta* reaches a very large size and the validity of its specific status should not be questioned because important morphological differences exist between this and the other *Moroteuthis* species (C. C. Lu, personal communication). The small specimen studied

here for morphometric analysis was not an adult and we speculate that the form was thus different from the adult and that it groups with other members of the genus because of its size. Thus we conclude this is an artefact. The differences between *M. robusta* and *M. ingens* mainly concern the skin structure and the number of hooks on the club. Kubodera *et al.* (1998) found that their specimens of *M. robusta* were close to *M. robsoni*; this was not confirmed by our morphometric results but is supported by the molecular study. *Moroteuthis robusta* is close to *M. ingens* and *M. robsoni* and, in spite of important morphological differences, is closer to these *Moroteuthis* species than to *M. knipovitchi*. The *M. ingens* studied came from the Falkland Islands and the Antarctic area. The specimen of *M. knipovitchi* was collected near the Antarctic peninsula.

Onychoteuthis compacta is a Hawaiian species supposed to be geographically and morphologically isolated from *O. banksii* and *O. borealijaponicus*. This species is included in the complex described in Hawaiian waters (Young & Harman 1987). Molecular analysis shows very close relationships between three of the Hawaiian species studied, but the fourth species (n.sp.) is more distant. There was no specimen of *O. compacta* available for morphometric study, although the results obtained with the other species revealed considerable variation between all

specimens of *Onychoteuthis* analysed, even if no cluster could be defined. It would be necessary to obtain sequences from the two best-known *Onychoteuthis* species (*O. banksii* and *O. borealijaponicus*) to evaluate the degree of differentiation between all the species of the genus in relation to the geographical variation.

In conclusion, two congruent clarifications about the phylogenetic relationships among onychoteuthids can be reached with the morphological and molecular analyses presented here: *M. knipovitchi* is distant from the other *Moroteuthis* species; *Onykia* (especially *O. rancureli*) is not closer to *Moroteuthis* than to *Onychoteuthis* and its taxonomical independent status seems to be confirmed.

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APPENDIX A

Table A1. *Characteristics of the specimens measured*

(BAS, British Antarctic Survey (Cambridge); NHM London, National History Museum of London; MNHN Paris, Museum National d'Histoire Naturelle de Paris; NSM Tokyo, National Science Museum of Tokyo; RSM, Royal Scottish Museum. *N* is the number of specimens.)

identification (and identifier)	<i>N</i>	maturity stage	cruise and capture date	geographic area	origin and identification no.
<i>Moroteuthis ingens</i> Smith, 1881 (id. Kubodera)	19	juveniles	<i>Albatross</i> 20/02/1988	52°40.2' S, 58°42.4' W 52°50.1' S, 58°50.8' W	BAS
	2	juveniles	<i>Albatross</i> 18/02/1988	52°49.8' S, 58°5.6' W 52°59.0' S, 59°15.5' W	BAS
	4	juveniles	October 1985	47° W, 40° S 12 nautical miles (22.2 km) east of Port Stanley	BAS
	2	juveniles	20/02/1987	53° S, 59° W	BAS
	1	—	21/11/1988	—	
	1	?			
	1	—	20/02/1988	52° S, 58° W	
	3	?		48°57.9' S, 60° W STN 20	BAS
	1	adult (F)	<i>Showa Maru</i> 23 16/03/1986	49°03.67' S, 60°37.4' W	BAS
	2	adults (F)	22/03/1996	Falklands STN 1	BAS
<i>Moroteuthis knipovitchi</i> Filippova, 1972	1	adult (F)	25/12/1970	St 54 S off New Zealand	NSM Tokyo 67464
	1	adult (F)	<i>Professor Siedlecki</i> STN 2	53°20' S, 42°42' W	BAS
	1	adult (F)	<i>Octos</i> 21/02/1985	61°24' S, 54°63' W STN 136	BAS
	1	adult (F)	02/04/1990	Signy Island station	BAS
	1	adult	01-02-1991	South Georgia Station 45 (Falklands)	BAS

(continued)

Table A1. (*continued*)

identification (and identifier)	<i>N</i>	maturity stage	cruise and capture date	geographic area	origin and identification no.
<i>Moroteuthis pacifica</i> Okutani, 1983 (id. Okutani)	1	adult (M)	<i>Ryun-Maru</i> 10/07/1979	40°51' N, 148°7' W	NSM Tokyo 61210 holotype
(id. Okutani)	1	adult	<i>Koei-Maru</i> 26/06/1978	35°54' N, 175°39' E	NSM Tokyo 61212 paratype no. 3
<i>Moroteuthis lönnbergi</i> Ishikawa & Wakiya, 1914 (id. Okutani)	2	adults	09/05/1979	Suruga bay	NSM Tokyo 67330
<i>Moroteuthis aequatorialis</i> Thiele, 1920 (id. Kubodera)	1	adult	02/02/1981	01°18.3' S, 84°46.7' W	NSM Tokyo 66825
<i>Moroteuthis robsoni</i> Adam, 1962 (id. Kubodera)	1	adult		41°16' S, 152°00' E	NSM Tokyo 67259
<i>Moroteuthis robusta</i> Dall in Verrill, 1876 (id. Kubodera)	1	adult	15/06/1980	37°45.0' N, 158°8.7' W	NSM Tokyo 67199
<i>Kondakovia longimana</i> Filippova, 1972 (id. Kubodera)	1	adult (F)	<i>Seishu Maru</i> 26	52°44.74' S, 47°02.37' W	BAS
	9	juveniles	<i>Banshu-Maru</i> 05/10/1984	42°3' S, 57°38' W	NSM Tokyo 63968
group Y (id. C. C. Lu)	10	?	04/06/1987	50°33' S, 62°01' W	BAS
<i>Onychoteuthis banksii</i> Leach, 1817	1	adult (M)	<i>John Biscoe</i> 23/09/1986	34°23.32 N, 15°10.85' W	BAS
	1	adult	1854	Cape of Good Hope	NHM London 1854.11.15.20
	1	adult (poor condition)	1958	Madeira	NHM London 1958.12.30.25
	1	adult	1885	Lake Gambia	NHM London 1885.2.11.1
	1	adult (F)	1890	between Tonga and Auckland	NHM London 1890.12.28.103
	1	?	?	?	NHM London 62.11.21.9
(id. W. J. Rees, 1947)	1	?	03/05/1934	?	NHM London
	1	?	<i>John Murray</i> exp.	?	NHM London
(id. F. Naggs)	1	?	30/01/1981	31° N, 125° W (to 129° W)	NHM London
	1	adult	1919	South of Madeira	NHM London 1919.12.30.47
(id. J. C. J. Galbaith, 1958)	1	adult (poor condition)	20/05/1957	Bay of Biscay 45°04' N, 4°48' W	NHM London 1958.6.10.1-8
(id. WJ Rees, 1947)	1	adult (M)	24/10/1929	between 38°09' S, 23°48' E 39°37' S, 25°29' E	NHM London
(id. Okada, 1925)	1	adult	1844	Bahia	MNHN Paris 3.1.637
(id. Okada, 1925)	1	adult	1830	Atlantic Ocean	MNHN Paris 3.1.627
(id. Okada, 1925)	1	adult	1834	Between Tropics	MNHN Paris 3.1.630
(id. Okada, 1925)	1	adult	1846	Mediterranean	MNHN Paris 3.1.635
(id. Okada, 1925)	1	adult (F)	1834	Atlantic Ocean	MNHN Paris 3.1.626
(id. Okada, 1925)	1	adult	—	Atlantic Ocean	MNHN Paris 3.1.633
(id. Okada, 1925)	1	juvenile	—	America	MNHN Paris 3.1.629
(id. Okada, 1925)	1	adult	—	Malabar	MNHN Paris 3.1.636
(id. Okada, 1925)	1	adult	1834	Eels Bank	MNHN Paris 3.1.631
(id. Okada, 1925)	1	adult	1841	Chile coast	MNHN Paris 3.1.634
(id. Kubodera)	4	adults (2M + 2F)	<i>Soyo-Maru</i>	32°09' N, 141°05' E	NSM Tokyo 21/07/1974 66890

(*continued*)

Table A1. (continued)

identification (and identifier)	N	maturity stage	cruise and capture date	geographic area	origin and identification no.
<i>Onychoteuthis bartlingii</i> Lesueur, 1821	1	adult	?	New Zealand	NHM London
<i>Onychoteuthis</i> sp.	2	adults (F, M)	12/1948	Mukalla, South Arabia	NHM London
	1	adult	—	Borneo	NHM London
	1	adult	?	?	NHM London
					68.6.8.6
<i>Onychoteuthis borealijaponicus</i> Okada, 1927	2	adults (M + F)	17/09/1927	38°22' N, 123°47' W	NSM Tokyo
(id. Kubodera)					66909
Onychoteuthid	1	?	<i>Templeton Crocker</i> expedition 26/04/19	23° N, 108°11' W	NHM London Station 152
<i>Ancistroteuthis lichtensteini</i> Férussac & d'Orbigny, 1839	1	adult	—	38°11' N, 15°33' E	1903.48.10 RSM
	1	adult (poor condition)	—	Mediterranean	NHM London
	2	adults	—	Nizza	45.9.26.25 NHM London
	1	adult (F)	—	Messina	NHM London
					45.9.8.1
(id. Okada, 1925)	1	adult	1841	Mediterranean	MNHN Paris
					3.1.622
(id. Okada, 1925)	1	adult	—	Nizza	MNHN Paris
					3.1.620 syntype
<i>Onychoteuthis lichtensteini</i> = <i>Ancistroteuthis lichtensteini</i>	1	adult	—	Mediterranean	NHM London
					73.4.6.4
	1	adult	1841	Mediterranean	MNHN Paris
					3.1.618
	1	adult	—	Nizza	MNHN Paris
					3.1.623
<i>Onykia rancureli</i> Okutani, 1981	2	adult (F)	<i>Kaki-Maru</i>	24°58.0' S, 108°59.0' W	NSM Tokyo
(id. Okutani)		and immature	26/10/1979	EP3	67275
<i>Onykia indica</i> Okutani, 1981	1	adult (M)	17/12/1995	12°50' S, 108°58' E	NSM Tokyo
(id. Okutani)					59494 paratype 1
(id. Okutani)	1	adult (F)	26/12/1995	11°34' S, 109°45.5' E	NSM Tokyo
					59493 holotype

APPENDIX B

Table B1. Specimens used for molecular analysis, their geographical origin and EMBL accession numbers

specimens		geographical origin	EMBL accession numbers
<i>Onykia</i> sp. (id. R. E. Young)	larvae	Hawaii	AJ223486
<i>Onychoteuthis compacta</i> (id. R. E. Young)	larvae	Hawaii	AJ223482
<i>Onychoteuthis</i> sp. B (id. R. E. Young)	larvae	Hawaii	AJ223481
<i>Onychoteuthis</i> sp. C (id. R. E. Young)	larvae	Hawaii	
<i>Onychoteuthis</i> sp. (id. R. E. Young)	larvae	Hawaii	AJ223483
<i>Moroteuthis ingens</i>	adults	Falklands, New Zealand	X79580
<i>Moroteuthis robsoni</i> (id. C. C. Lu)	adult	New Zealand	AJ223487
<i>Moroteuthis knipovitchi</i> (id. M. Vecchione)	adult	South Shetland Islands, Antarctica	AJ223488
<i>Moroteuthis robusta</i> (id. S. O'Shea)	adult	New Zealand	AJ223489
<i>Enoploteuthis higginsii</i> (id. R. E. Young)	larvae	Hawaii	AJ223485
<i>Enoploteuthis galaxias</i> (id. L. Bonnaud)	adult	New Zealand	AJ223484

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