The genetics and taxonomy of Southern Ocean Octopodidae, with special reference to the genus *Pareledone*.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Anne Louise Allcock.

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Chapter 1

A general introduction to the taxonomy of the Octopodidae with special reference to the Southern Ocean.

The genetics and taxonomy of Southern Ocean Octopodidae, with special reference to the genus *Pareledone*.

A Louise Allcock

Abstract

The Octopodidae of the Southern Ocean have been afforded little attention since the early part of this century and their taxonomy is poorly known. During this study, three extensive trawling programmes took place off South Georgia, off the Antarctic Peninsula and in the Weddell Sea. A further collecting programme was arranged in the Falkland Islands, just north of the Antarctic Polar Front. Twenty-six putative species were recorded from the Southern Ocean; there are just sixteen previously described species from this region.

Allozyme electrophoresis was used to validate the specific status of samples. A dendrogram was constructed from genetic identity values. Ten species of *Pareledone* clustered together suggesting that the genus is valid. It has been noted that members of this genus have only plesiomorphic and no apomorphic characters in common, hence there was previously no evidence that *Pareledone* species share a unique evolutionary history. Three species of *Pareledone* clustered separately, but it has already been suggested that these species should be removed from the genus on clear morphological grounds. The dendrogram provided evidence that supported the use of sucker serialisation as a dichotomous character to subdivide the family Octopodidae, but refuted the use of the presence / absence of an ink sac as a subfamilial character.

The use of morphological measurements in octopodid taxonomy was tested using multivariate statistical techniques. When a large suite of characters was measured there was satisfactory discrimination between species. Counts of sucker numbers and gill lamellae were particularly useful for discrimination at the specific level, but phenograms constructed from analyses of these and other measurements appeared to be of little phylogenetic use. The shape and size of the beak were found to be more useful at the generic level. A phenogram constructed from analyses of beak morphology bore some resemblance to a dendrogram constructed from genetic identity values. Analysis of more genera is required though before it can be asserted that beak structure is useful in reconstructing octopus phylogenies.

On the basis of electrophoretic and morphological analyses, seven new species of the genus *Pareledone* are described. The diagnosis of the genus is amended to exclude "*Pareledone*" *polymorpha* and "*Pareledone*" *adeliana* and two new similar species. A new genus should be constructed for these four species; a suggested diagnosis for this new genus is given. Specimens of rare and unusual genera were also obtained. Short descriptions of each of these are given, but these are the subject of ongoing work.

Maps are provided of the locations from which specimens were obtained during this study. Although the trawling programmes were extensive there is evidence from the catch data that not all the species extant in the sample area were captured. This is especially true in areas of deeper water.

The genetic structure of stocks of *Pareledone turqueti* around South Georgia and Shag Rocks was investigated. *Pareledone* species have large eggs, which probably give rise to large, crawl-away young. The extreme heterogeneity found between two populations was attributed to the limited dispersal of these benthic hatchlings. The probable inability of *Pareledone* species to maintain panmixia over large areas is discussed with respect to the radiation of the genus.

Taxonomic history of the Octopodidae

Devotion to the study of octopuses dates back as far as Aristotle who described, with great care, the features and behaviour of many Mediterranean forms. Modern literature on the group dates from the seventeenth century, but it was not until 1835, when d'Orbigny and de Férussac published the first section of their "Histoire Naturelle des Céphalopodes acétabulifères", that a serious attempt was begun to construct an exhaustive classification for the group. In 1845 d'Orbigny erected the family Octopodidae for the benthic octopods.

Whilst authors such as Hoyle, Joubin, Massy, Berry and Verrill continued to produce detailed studies of both new and previously described Octopodidae, it was left to Gray, d'Orbigny and Grimpe to produce a useable scheme for their systematics. They were joined in the 1920s by Robson who began his "Monograph of the recent Cephalopoda". It was a mammoth task that had as its object "a complete study of the morphology and evolution of the Class Cephalopoda". Although it contains "odd, often conflicting, and many times erroneous, statements" (Voss, 1977), apparently as a result of the mental difficulties from which Robson was suffering at the time, it was an invaluable attempt to draw together all the existing knowledge of several families, including the Octopodidae, and to introduce order into the growing mass of species.

The classification set out in Part I of his monograph (Robson, 1929) divides the family Octopodidae into three subfamilies on the basis of presence/absence of an ink sac, sucker serialisation and egg size (Table 1.1). Whilst neat and orderly, the simplicity of this classification was clearly bothering Robson by the time he published Part II of his monograph (1932). Recognising the loss of an ink sac as an adaptation to depth, Robson noted that genera such as *Benthoctopus*, whilst showing some "abyssal" characteristics, "nevertheless retain traits that ally them with true *Octopus*", and accordingly placed the genera *Benthoctopus, Teretoctopus* and *Grimpella* in the subfamily Octopodinae (Table 1.2). In addressing the subfamily Bathypolypodinae he was faced with the problems of the genera *Graneledone, Bentheledone* and *Thaumeledone* which, whilst possessing many "abyssal" traits in

Table 1.1: Classification of the order Octopoda illustrating the position of the family Octopodidae and the subdivisions within that family. Reproduced from Robson, 1929.

Order	Suborder	Family	Subfamily	Diagnosis
OCTOPODA, Leach				
	1. CIRRATA, Grimpe			
	2. PALAEOCTOPODA, Naef			
	3. INCIRRATA, Grimpe			
		1. ELEDONELLIDAE, Sasaki		
		2. AMPHITRETIDAE, Hoyle		
		3. OCTOPODIDAE, d'Orbigny		
			1. ELEDONINAE, Grimpe	ink sac present eggs large suckers uniserial
			2. OCTOPODINAE, Grimpe	ink sac present eggs usually small suckers biserial
			3. BATHYPOLYPODINAE, Robson	ink sac absent eggs (?) [sic] suckers biserial
		4. ARGONAUTIDAE, Naef		

Table 1.2: Ammendments to Robson's (1929) classification of the Octopodidae, according to Robson, 1932. ?, status of genus is doubtful in Robson's opinion; *, information not available from Robson's descriptions; †, genus erected since Robson's 1929 classification.

Subfamily	Genus	Number of suckers rows	Presence of ink sac
I. OCTOPODINAE			
	Benthoctopus	2	×
	Teretoctopus †	2	×
	Grimpella	*	×
?	Haptochlaena	*	*
	Eledone	1	✓
	Pareledone †	1	✓
?	Eledonenta †	1	✓
	Veledona	1	✓
II. BATHYPOLYPODINAE			
	Bathypolypus	2	×
	Graneledone	1	×
	Thaumeledone †	1	×
	Bentheledone †	1	×

common with *Bathypolypus,* are divergent in the arrangement of suckers. Naef (1923) had previously expressed mistrust as to the taxonomic value of sucker arrangement and, based on this, had doubted the validity of the subfamily Eledoninae and suggested it may not be "monotypic". Clearly, by 1932, Robson concurred and he reduced the number of octopodid subfamilies to two, placing *Eledone, Pareledone, Eledonenta* and *Velodona* in the Octopodinae, and *Graneledone, Thaumeledone* and *Bentheledone* in the Bathypolypodinae (Table 1.2).

Further comment on the classification of the Octopodidae as a whole was not made until the 1970s. Voss (1977) published a classification of the entire Class Cephalopoda and, on reviewing the status of cephalopod systematics, wrote "what a sad state of affairs exists in this field". In 1988, Voss erected a new subfamily, the Graneledoninae, based on two of the characteristics (ink sac and sucker arrangement) that Robson had previously deemed unsuitable, and hence provided an alternative classification of the Octopodidae (Figure 1.1, Table 1.3). This classification, as the most recent, is perhaps the most widely used, and as such will be the classification used throughout this thesis.

Problems of Octopodidae taxonomy

Correctness is not a corollary of either usage or acceptance and criticisms of Voss' (1988a) classification are widespread. Voight (1993a) provided data based on sucker morphology that indicate the subfamilies constitute phenetically similar, rather than monophyletic, groups and has suggested that a complete reassessment of octopod phylogeny is required. Indeed, cladistic treatment of Voss' data (Voight, 1993b) produced a phylogeny that differed widely from that of Voss (Figure 1.2). Furthermore, members of the genus *Aphrodoctopus* can possess both biserial and uniserial configurations of suckers on the same specimen (Roper and Mangold, 1991), an observation which throws further doubt on the validity of ascribing genera to subfamilies according to sucker serialisation. Although there is little published criticism on the use of the ink sac as a taxonomic character, if Robson (1932) is

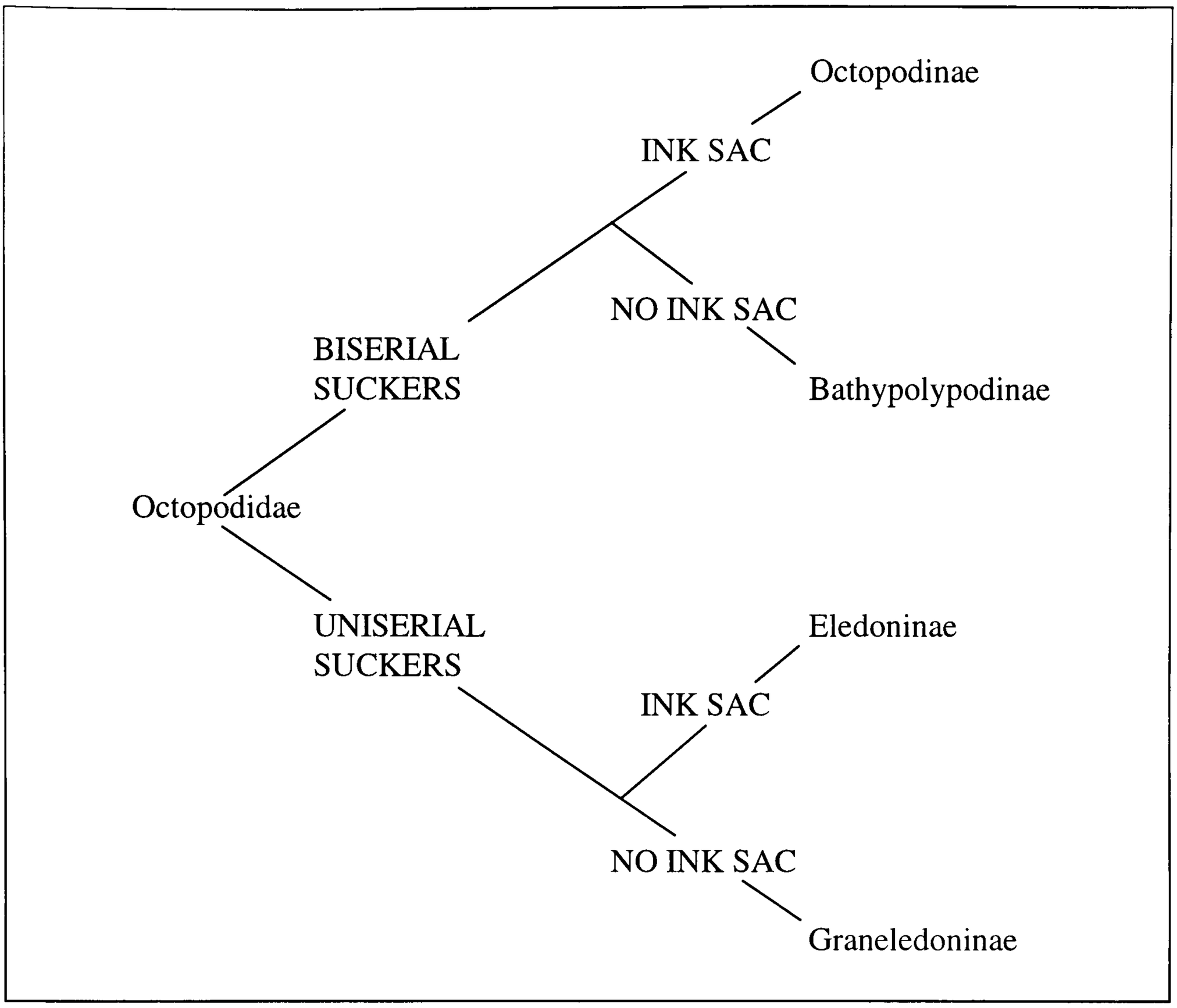


Figure 1.1: Taxonomic characters that currently distinguish the sub-families of the Octopodidae. Adapted from Voss (1988a).

Table 1.3: Current placement of genera within octopodid subfamilies. Compiled from Voss (1977, 1988a, b) and Toll (1991). *, although Voss ignores this genus most authors consider it to be valid (Kubodera and Okutani, 1986, 1994; Lu and Stranks, 1994); ?, status of genus is questionable.

Subfamily	Genus	
Octopodinae	*Octopus*	
	Enteroctopus	
	Cistopus	
	Robsonella	
	Scaeurgus	
	Pteroctopus	
	Hapalochlaena	
	Euaxoctopus	
	Macrotritopus	
	Aphrodoctopus	?
	Macroctopus	?
	Callistoctopus	?
Eledoninae	*Eledone*	
	Pareledone	
	Megaleledone	*
	Tetracheledone	
	Vosseledone	
	Velodona	
Bathypolypodinae	*Bathypolypus*	
	Benthoctopus	
	Teretoctopus	
	Grimpella	?
Graneledoninae	*Graneledone*	
	Thaumeledone	
	Bentheledone	

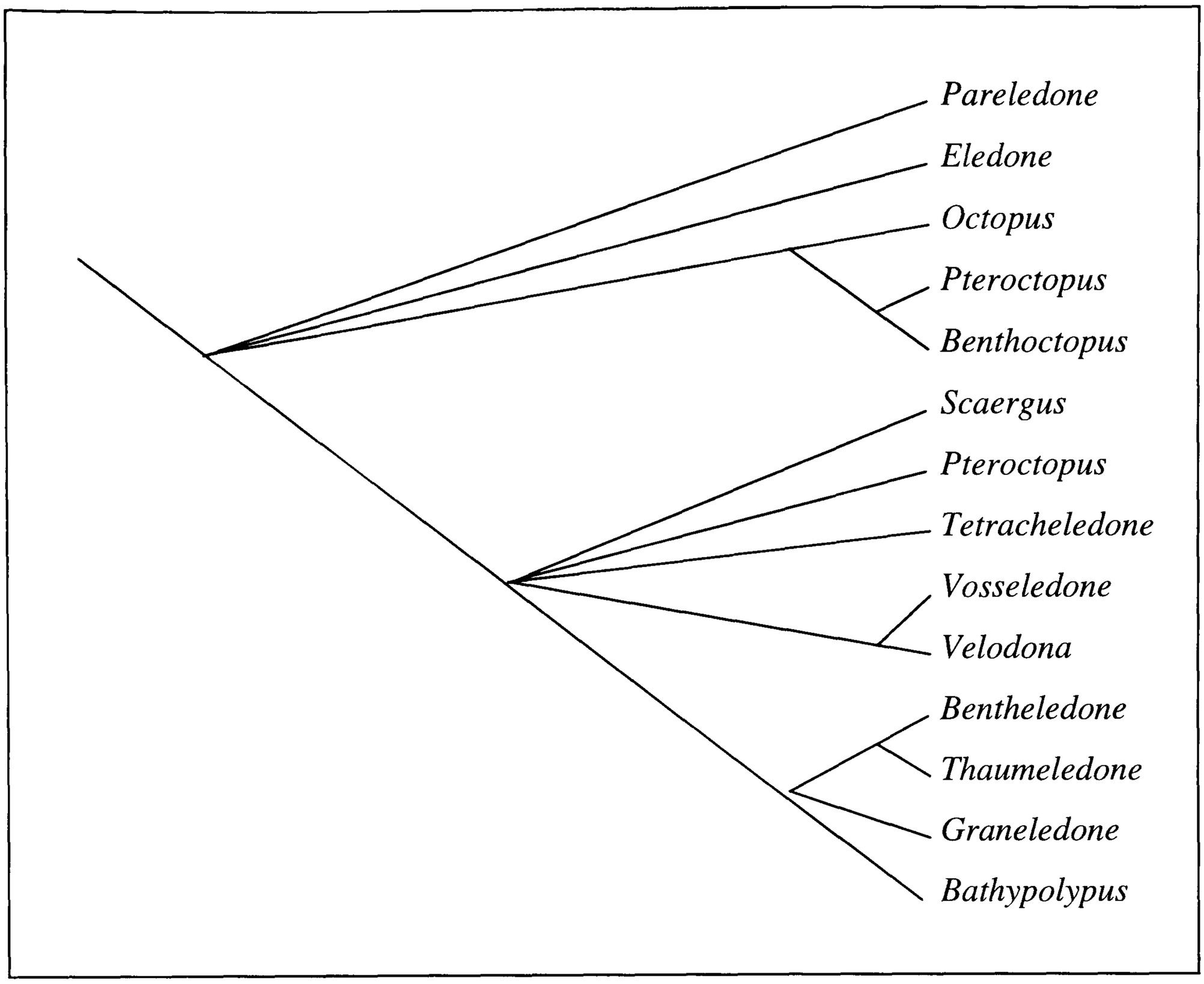


Figure 1.2: Voight's (1993b) octopodid phylogeny based on cladistic reassessment of Voss' data.

correct, and loss of the ink sac is an adaptation to depth, then it is highly likely that this condition could have arisen on many different occasions. Hence a group simply containing species without ink sacs is likely to be polyphyletic.

Many of the problems of octopodid phylogenetics arise because of the phenotypic plasticity of the animals and because of the lack of hard structures that can be used as taxonomic characters. The beak and the radula are the only hard anatomical structures traditionally used in octopodid classification and whilst there has been extensive characterisation of these features in specific descriptions (Lu and Stranks, 1991; Norman, 1991; Villanueva *et al.*, 1991) their taxonomic importance has seldom been reviewed (but see Adam, 1941; Clarke, 1962). Although it appears that beaks may be useful at the generic level (F. G. Hochberg, pers. comm., 1994), Voss (1977) states that the "illustration of only a single radula would possibly confuse generations of later workers".

Out of necessity many soft tissues are also used as diagnostic taxonomic characters (Roper and Voss, 1983). Although it has been proposed that it is the use of ratios (see Strauss, 1985) in cephalopod taxonomy that has led to the assumption of excessive morphological variation (Voight, 1991) and that in fact the external morphology of cephalopods is sufficient to provide a basis for taxonomy, the phenotypic plasticity of the morphology of octopod soft parts cannot be ignored (Voss, 1977). Furthermore, many forms of preservation lead to increased morphometric variability. These effects have been shown to be significant in squid (Andriguetto and Haimovici, 1988) where they widen the confidence limits of most indices. Clearly if soft parts are to be used as characters then measurements should ideally be taken from specimens before they are preserved or frozen and, whenever possible, they should also be taken from a large number of individuals. Even so, Voight (1994) states that "characters of shape, deemed essential to species descriptions, do not appear to be able to identify most specimens, nor to increase our understanding of octopus biology."

The Octopodidae of the Southern Ocean

The taxonomy of the octopodids of the Southern Ocean is in a particularly unsettled state. Whilst, in part, this is a reflection of the general need for systematic revision of the group, the problem is exacerbated by environmental conditions. The majority of species live in deep water and much of the ocean is covered with sea ice, necessitating the use of specialised ships and limiting the time of collecting expeditions. As a consequence many Southern Ocean species were described on the basis of only a single specimen and some are yet to be recaptured.

Sixteen species of Octopodidae are currently recognised from the Southern Ocean (Table 1.4). These represent six genera and, following the classification of Voss (1988a), three subfamilies.

The most abundant and diverse genus is *Pareledone* of the subfamily Eledoninae. The genus is diagnosed as having small, uniserial suckers, a well developed web, well developed gills with 6-9 lamellae, a VV or W shaped funnel organ, an ink sac, a well developed crop and no cartilaginous stylets. The status of this genus has been questioned as rather than being a group of species that share a unique evolutionary history it is a group that simply shares primitive character states (Voight, 1993b). The genus is endemic to the Antarctic and species are generally found only south of the Polar Front. Although specimens have been caught further north, (e.g. *P. turqueti* recorded off Brazil by the Terra Nova Expedition; Massy, 1916), they have always been taken in Antarctic Deep Water and Voss (1988b) suggests that temperature is the factor controlling their biogeography. Alternatively it has been suggested that the Brazilian "*Pareledone*" specimens are conspecific with either *Eledone massyae* Voss, 1964 or *E. gaucha* Haimovici, 1988 (F. G. Hochberg, pers. comm., 1994).

A recent review of this genus was published by Lu and Stranks (1994) following extensive fishing in eastern Antarctic waters. Of twelve species described in total, three have been synonymized (Table 1.4) and two others, *P. carlgreni* Thore, 1945 and *P. nigra* (Hoyle, 1910) (neither of which is found in the Southern Ocean),

Table 1.4: Octopodidae currently recognised from the Southern Ocean

Subfamily	Genus	Species	junior synonym
Eledoninae	*Pareledone*	*charcoti* (Joubin, 1905)	
			aurorae (Berry, 1917)
		harrissoni (Berry, 1917)	
			antarctica (Thiele, 1920)
		turqueti (Joubin, 1905)	
		prydzensis Lu and Stranks, 1994	
		framensis Lu and Stranks, 1994	
		adeliana (Berry, 1917)	
			umitakae Taki, 1961
		polymorpha (Robson, 1930)	
	Megaleledone	*senoi* Taki, 1961	
Graneledoninae	*Graneledone*	*setebos* Robson, 1932	
		antarctica Voss, 1976	
	Bentheledone	*albida* (Berry, 1917)	
		rotunda (Hoyle, 1885)	
	Thaumeledone	*gunteri* Robson, 1930	
		brevis (Hoyle, 1885)	
Bathypolypodinae	*Benthoctopus*	*levis* (Hoyle, 1885)	
		thielei Robson, 1932	

are being systematically reviewed by Roper and Mangold, who consider that they should be placed in *Aphrodoctopus*; this leaves seven valid species.

P. framensis and *P. prydzensis* are both recently described (Lu and Stranks, 1994). Both appear to be restricted to eastern Antarctica (Figure 1.3a), the former species being found on the Fram Bank at depths of 145-319 m, the latter at Prydz Bay at depths of 526-676 m.

P. harrissoni appeared to be restricted to eastern Antarctica (Figure 1.3a) where it is found in depths of 25-743 m (Lu and Stranks, 1994), although there is one report of its presence off the Antarctic peninsula (Kubodera and Okutani, 1994). Voss (1988b) designated *P. harrissoni* a *nomen dubium* without giving reasons. Lu and Stranks (1994) confirm its validity and concur with Robson's (1932) suggestion that *P. antarctica* is a junior synonym of this species.

P. turqueti is similar to *P. harrissoni* and Dell (1959) and Voss (1988b) consider it to be circum-Antarctic and found in depths of 0-1,000 m. The conclusion of a taxonomy workshop at the 1992 Southern Ocean Cephalopod Symposium, in Cambridge, UK was, however, that this species is probably restricted to western Antarctic waters (Figure 1.3a).

P. charcoti has been found in depths down to about 700 m and is the only circum-Antarctic species described from this genus (Figure 1.3a) (Lu and Stranks, 1994). Two forms of the species have been noted which differ in the shape of the tubercles on the dorsal surface. Robson (1930, 1932) comments that Joubin's earlier (1905) description deals with a "heavy sculpture of closely opposed boss-like tubercles" whilst his later (1914) paper describes "a more granular type of sculpture, the tubercles being smaller and more widely spaced". Berry's (1917) description of *P. aurorae* is very similar to Joubin's 1914 description of *P. charcoti* and Berry himself (1918) later designated the former a junior synonym of the latter. Kubodera and Okutani (1994) conclude that "specimens fixed while alive have distinct tubercles...identical to the form seen in the type of *P. charcoti*" whereas "specimens

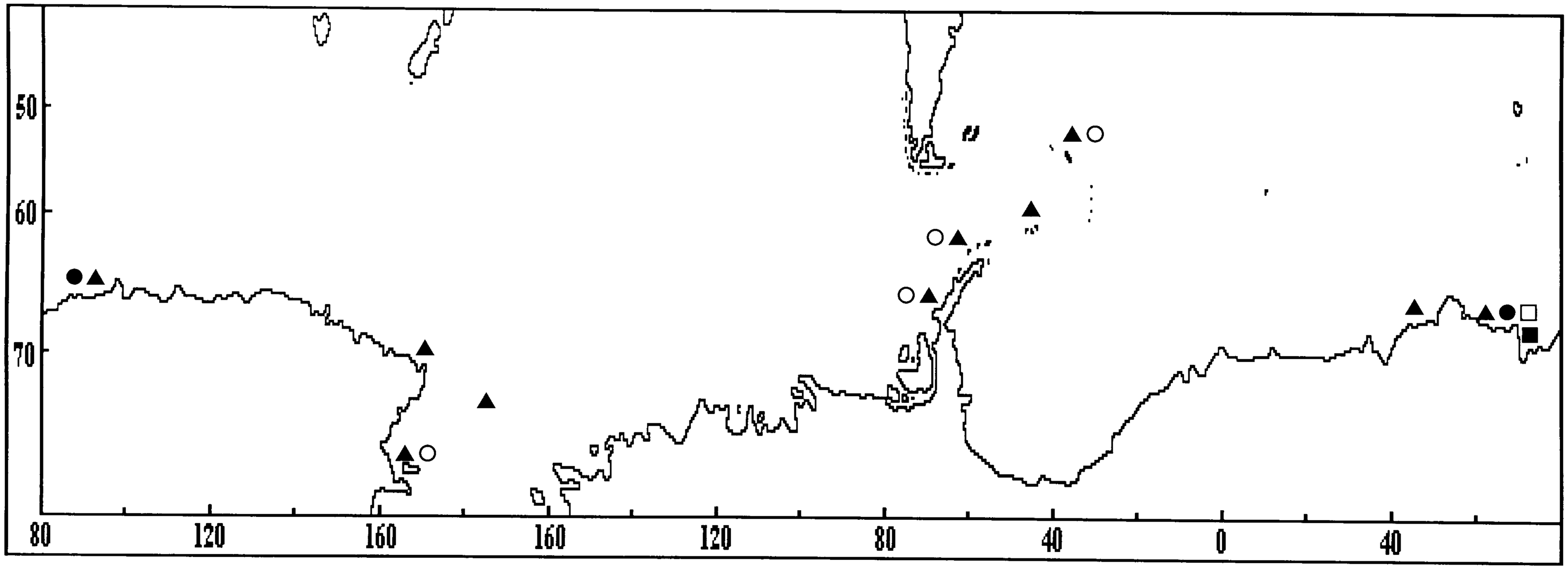


Figure 1.3a: Recorded distribution of Southern Ocean Octopodidae. Eledoninae.

■ = *P. framensis*. □ = *P. prydzensis*. ● = *P. harrissoni*. ○ = *P. turqueti*. ▲ = *P. charcoti*.

(Sources: Robson, 1932; Kuehl, 1988; Lu and Stranks, 1994)

fixed when dead...tend to have low, indistinct tubercles...found in the type of *P. aurorae*". They concur with Berry that the two species are synonymous.

P. polymorpha (from western Antarctica) and *P. adeliana* (from eastern Antarctica, though see Kubodera and Okutani, 1994) (Figure 1.3b) are similar to one another but differ markedly from the other *Pareledone* species. They have smaller, differently shaped beaks, larger grooved hectocotyli and different patterns of chromatophores on the viscera. A third similar species, *P. umitakae* has been synonymized with *P. adeliana* (Lu and Stranks, 1994) on the basis of Taki's (1961) description. Lu and Stranks (1994) suggest that removal of the two valid species (*P. polymorpha* and *P. adeliana*) from the genus *Pareledone* may be justified on the basis of the differences stated above. To indicate the uncertainty surrounding their generic status they are referred to in this thesis as "*Pareledone*".

The other Antarctic species belonging to the Eledoninae is *Megaleledone senoi* which is distributed in the Indo-Atlantic sector of the Southern Ocean from depths of 120-803 m (Figure 1.3b). Taki (1961) erected a new genus and subfamily for this species. Voss (1988b) considered the genus to be invalid and refers to *Pareledone senoi*. Alternatively, however, the genus may be valid although the subfamily is not (Kubodera and Okutani, 1986, 1994; Lu and Stranks, 1994). Taki (1961) notes the similarity of this species to *Graneledone setebos*, which is known from a single mangled specimen. It is likely that these species are synonymous (F. G. Hochberg, pers. comm., 1995) and if this is confirmed the correct name of the species would become *Megaleledone setebos* (Robson, 1932).

All three genera of the subfamily Graneledoninae have Southern Ocean representatives (Figure 1.4). The genus *Graneledone* is diagnosed as having uniserial suckers, no ink sac, a VV shaped funnel organ, a reduced or no crop, small gills, a small hectocotylus and mantle and arms covered with small to large cartilaginous spiny warts. Although the genus is not confined to the Southern Ocean, two species within the genus are. The first, *Graneledone setebos*, is mentioned above. The other, *Graneledone antarctica*, was described from immature males and females taken from

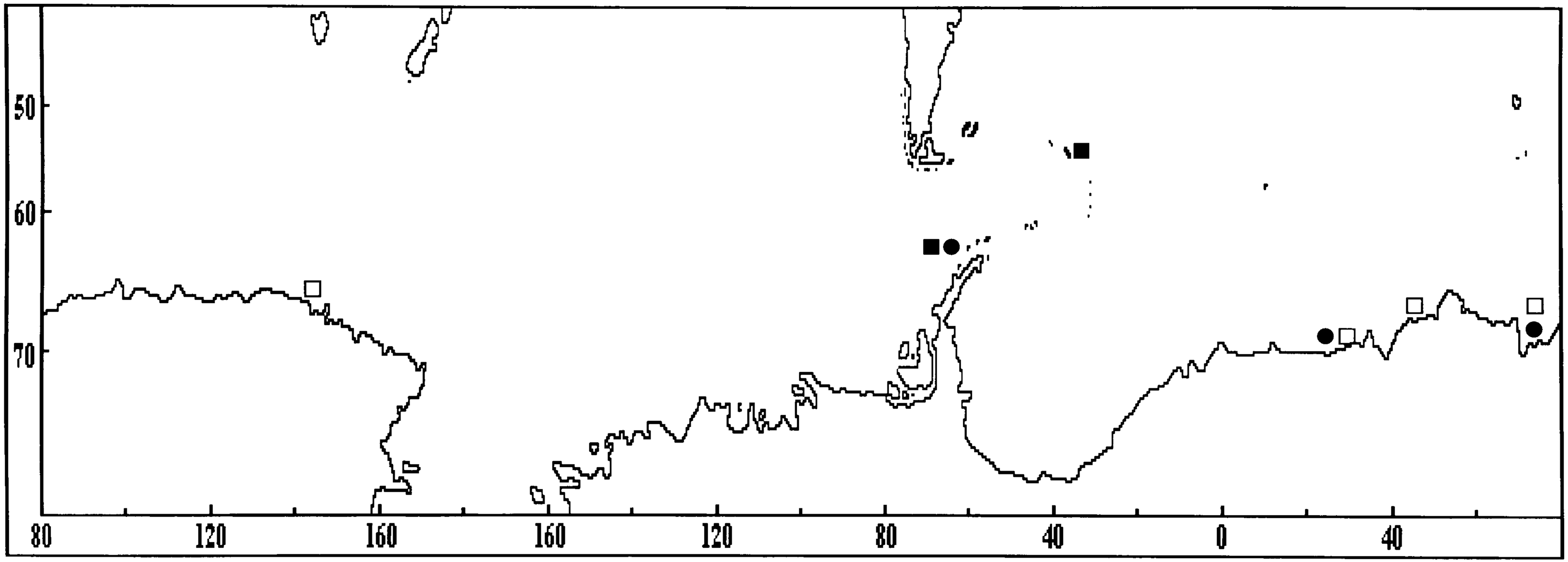


Figure 1.3b: Recorded distribution of Southern Ocean Octopodidae. Eledoninae (continued).

■ = *P. polymorpha.* □ = *P. adeliana.* ● = *M. senoi*

(Sources: Robson, 1932; Taki, 1961; Kubodera and Okutani, 1986; Kuehl, 1988; Lu and Stranks, 1994)

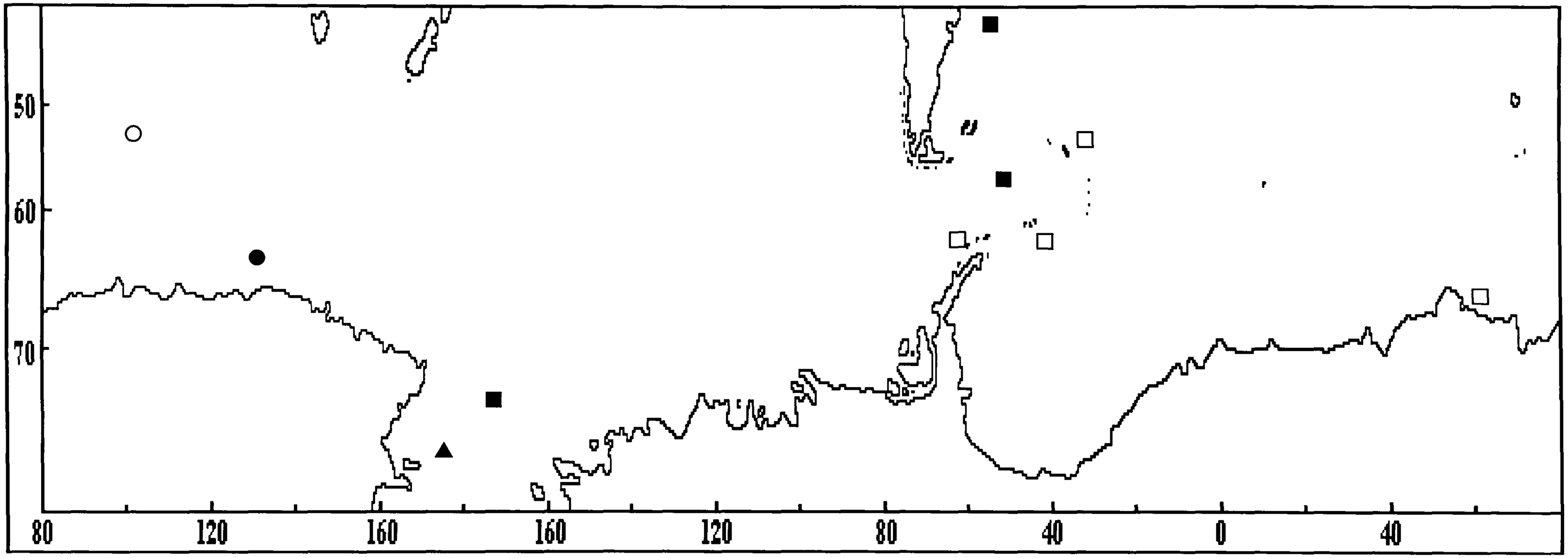


Figure 1.4: Recorded distribution of Southern Ocean Octopodidae. Graneledoninae.
▲ = *G. antarctica*. □ = *T. gunteri*. ■ = *T. brevis*. ○ = *B. rotunda*. ● = *B. albida*.
(Sources: Robson, 1932; Voss, 1976; Stranks *et al.*, in prep.)

2,341 m at 74°05.6'S 175°05.2'W (Voss, 1976). This is the only record of this species.

Both the genera *Bentheledone* and *Thaumeledone* are confined to the Southern Ocean. *Bentheledone* is described as having short arms, a deep web, no crop, no ink sac, small gills and a V shaped funnel organ. *Bentheledone rotunda* is described from one female specimen caught east of Kerguelan at 3,600 m and *B. albida* is also known only from the type specimen which was caught off Wilkes Land, Antarctica at 3,100 m. The latter has slightly longer arms than *B. rotunda*. The descriptions of both species are poor and should other specimens be captured these species need to be redescribed. Voss (1988b) mentions two other undescribed species of *Bentheledone* in his collections but gives no further information.

Thaumeledone, also comprising two species, is diagnosed as having a degenerate radula, small gills, a deep web and short sub-equal arms. *T. brevis* was first described from 1,100 m off Monte Video, but more recently has been shown also to be distributed around the Falkland Islands and in the Ross Sea at depths from 800-3,900 m. *T. gunteri* was originally described from a single female specimen taken in 400 m north east of South Georgia and is now known to reach as far as 66°S in depths of 400-900 m. The genus and both species are currently being redescribed (Stranks *et al.*, in prep.).

Of the subfamily Bathypolypodinae, only one genus, *Benthoctopus*, has representatives in the Southern Ocean (Figure 1.5). *B. levis* was described from 137 m depth off Heard Island and *B. thielei* was described from the shore of Kerguelan. Both descriptions are poor, and even the diagnosis of the genus is unclear. The entire genus, which comprises approximately fifteen species world wide, is in need of re-evaluation (M. Vecchione, pers. comm., 1996). *Benthoctopus* specimens were also reported from Elephant Island, South Shetlands (Kuehl, 1988) but these have not yet been identified to species level.

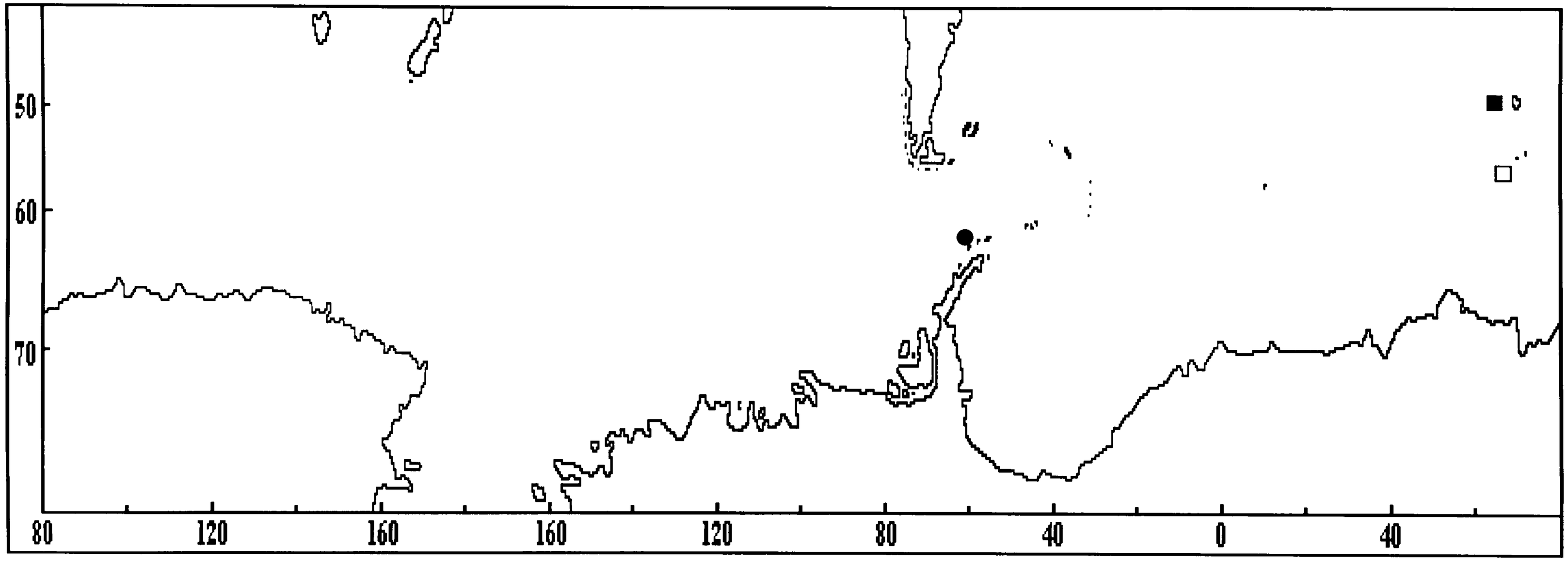


Figure 1.5: Recorded distribution of Southern Ocean Octopodidae. Bathypolypodinae.
□ = *B. levis*. ■ = *B. theiei*. ● = *Benthoctopus* sp.
(Sources: Robson, 1932; Kuehl, 1988)

The Octopodidae of the Falkland Islands and western Scotia Sea

Three species of Octopodidae have been described from the Falkland Islands in the South West Atlantic (Figure 1.6). *Benthoctopus eureka* (Robson, 1929) is a species that is only known from the Falkland Islands (Voss, 1988b). Although attributed to Robson, who classified the species as *Enteroctopus eureka*, the first specimen was probably seen by Hoyle in 1912 and misidentified as *Polypus tehuelchus*. *Octopus tehuelchus* d'Orbigny, 1835 and *Enteroctopus megalocyathus* (Gould, 1852) of the subfamily Octopodinae have also been reported from the area. Their distribution extends along the continental shelf from southern Brazil towards Patagonia. A fourth species, *Graneledone macrotyla* Voss, 1976 was reported from the West Scotia Basin in depths of 800-2,000 m (Figure 1.6). It is highly distinctive with large cartilaginous tubercles and is now known to extend from 45°S to 54°S (Kubodera and Okutani, 1994).

Protein electrophoresis as an aid to octopodid taxonomy

Undoubtedly conventional taxonomy could provide information on the species mentioned above, if new and fresh specimens were made available. However, fresh tissue samples would allow more modern biochemical and molecular techniques to be used alongside the conventional methods.

Protein electrophoresis is among the most cost-efficient methods of investigating genetic phenomena at the molecular level (Murphy *et al.*, 1990). It facilitates the measurement of genetic diversity between samples and has been used extensively to investigate phylogenetic relationships in marine organisms (Ward, 1989).

The only genetic study on octopodids to date confirmed two sympatric eledonids as valid biological species (Levy *et al.*, 1988) and led the authors to suggest the further use of electrophoresis for elucidation of the evolutionary pathways and radiation of this genus. The technique has been used extensively in

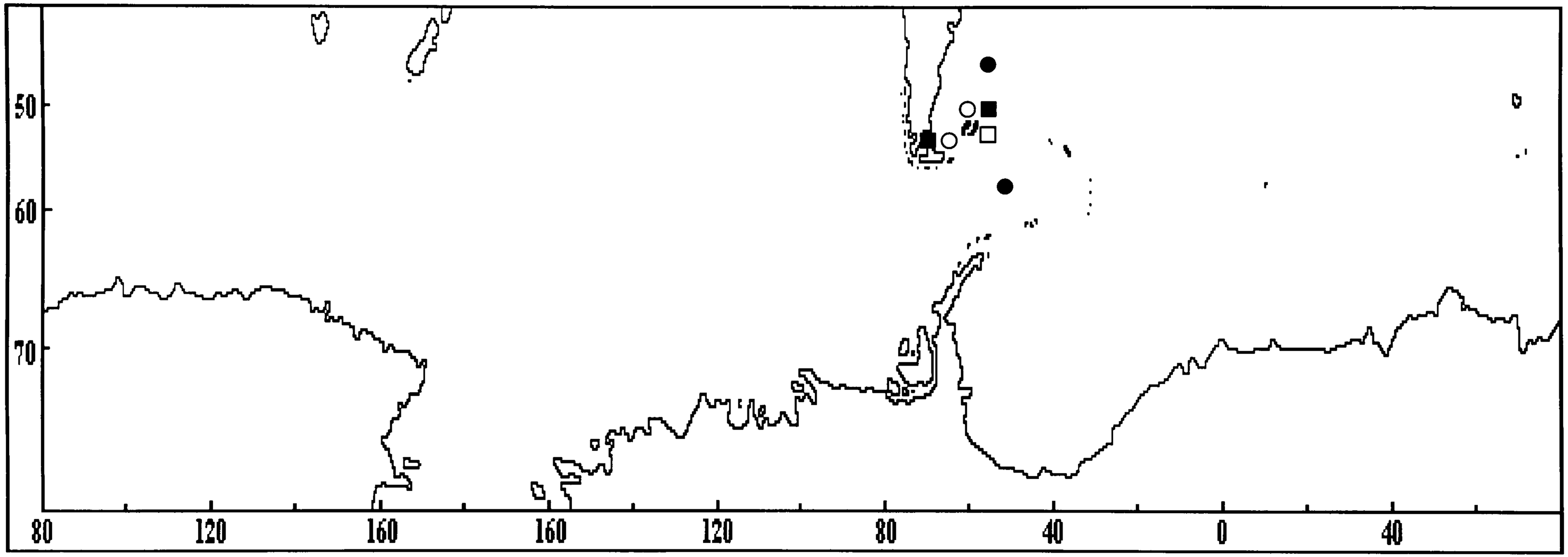


Figure 1.6: Recorded distribution of western sub-Antarctic Octopodidae.

□ = *Benthoctopus eureka*. ■ = *Enteroctopus megalocyathus*. ○ = *Octopus tehuelchus*. ● = *Graneledone macrotyla*.

(Sources: Robson, 1929; Voss, 1976; Kubodera and Okutani, 1994)

squid systematics (e.g., Brierley and Thorpe, 1994; Yokawa, 1994; Brierley *et al.*, 1996)

Electrophoresis has also led to the resolution of several problems in squid population biology (Thorpe *et al.*, 1986; Carvalho *et al.*, 1992; Brierley *et al.*, 1993b) illustrating the application of the technique on a finer scale. It is a valuable tool that provides relatively unbiased data regarding the genetic structure of stocks (Ihssen *et al.*, 1981; Ryman and Utter, 1987) and which allows the detection of effects such as inbreeding, non-random mating, and the pooling of genetically differentiated assemblages (Wahlund effect). It is potentially suitable for assessing the genetic structure of octopodid species over a range of geographically separated populations to give an insight into the extent of intraspecific morphological variation, plasticity and crypsis - a phenomenon that is becoming increasingly emergent amongst cephalopod species (Smith *et al.*, 1987; Yeatman and Benzie, 1993).

Sampling opportunities during this study

Throughout this study samples were collected from around the Falkland Islands by fishery observers on commercial vessels. These specimens were frozen and conveyed to the UK aboard British Antarctic Survey vessels.

There were three opportunities to collect specimens from the Antarctic: a demersal fish survey cruise around South Georgia by *MV Cordella*, a benthic survey of the Weddell Sea by *PFS Polarstern*, and a krill, fish and cephalopod survey of the Antarctic Peninsula by *PFS Polarstern.* The latter took place from November 1996 to January 1997 and initially it was not intended to include the information from that cruise in this thesis. Because, however, of the richness and diversity of octopus fauna found on the Antarctic Peninsula, tissue samples were carried back in a thermally insulated box for electrophoretic analysis and detailed measurements and drawings were made aboard ship for all specimens thought to belong to the genus *Pareledone*. Formalin fixed samples from this cruise have not yet arrived in the UK

and therefore discussion on specimens that do not fall within the genus *Pareledone* is reserved for later publications (e.g., Vecchione *et al.*, in prep).

Because of the confusion surrounding Southern Ocean octopus taxonomy each putative species was assigned a reference number as well as, where possible, a name. Species that are, as yet, unnamed, are referred to in this thesis by that reference number, whereas named species are always referred to by their Latin binomial.

Aims of the project

The aims of any project that relies on obtaining samples from an environment as inhospitable as the floor of the Southern Ocean must undoubtedly be broad. As taxonomy is fundamental to all biology, the primary aim was to make a contribution to Southern Ocean octopodid taxonomy using biochemical techniques (Chapter 2) and traditional taxonomy (Chapter 3), giving special attention to the genus *Pareledone* which, in biomass terms, is the major octopodid group in the area. It was always likely that undescribed species would be captured from the Southern Ocean and the evaluation of the taxonomic position of these species (Chapters 2 and 3) and their subsequent description (Chapter 4) was also considered of primary importance. A secondary aim was to produce a clear picture of the biogeography of the species captured (Chapter 5). Finally it was hoped that in some species it would be possible to study the population ecology (Chapter 6) to facilitate comments on the radiation of Southern Ocean octopodid species (Chapter 7).

Chapter 2

Taxonomic studies on Octopodidae from the Southern Ocean with special reference to the genus *Pareledone*: a biochemical genetic perspective.

Introduction

Antarctic octopodids have been insufficiently studied and their taxonomy is poorly known (Lu and Stranks, 1994). Recent benthic surveys of the Southern Ocean have revealed numerous apparently new species, many of which appear to belong to the genus *Pareledone*, which is endemic to the Southern Ocean. Since much confusion already surrounds the recognised species (see Chapter 1) it was felt that a biochemical technique, such as enzyme electrophoresis, should be used to clarify the status of both the existing and the putative new species.

Protein electrophoresis is based on the differential migration of water soluble protein molecules through supporting media under the influence of an electrical field. Migration rate is determined by various factors, including the charge, size and shape of the protein molecules, the pore size of the electrophoretic medium and the strength of the electrical potential (Murphy *et al.*, 1990). Using histochemical techniques (e.g. Harris and Hopkinson, 1987) the protein variants coded by a genetic locus (allozymes) can be stained as bands on the supporting media. Since allozymes behave in a straightforward Mendelian fashion (Avise, 1994) they are interpretable as simple allelic products of a gene. This allows calculation of allele frequencies at loci and thus the measurement of overall genetic diversity between groups of samples using established indices of genetic similarity, identity or distance (e.g. Rogers, 1972; Nei, 1972, 1978).

Genetic distance / identity is related to taxonomic separation by the molecular clock hypothesis (Zuckerlandl and Pauling, 1965; Fitch, 1976; Kimura, 1983; Hartl and Clark, 1989) which is based on the divergence of homologous proteins being proportional to evolutionary time since the rate of amino acid substitution is approximately constant. Thorpe (1982), using published data on genetic identity (I) concluded that a clear relationship exists between I and taxonomic divergence and that a critical value of I=0.85 delineates between con and non-specifics (Figure 2.1). Furthermore, if two sympatric putative species have no alleles in common at a single locus, then this locus is considered to be diagnostic for those species.

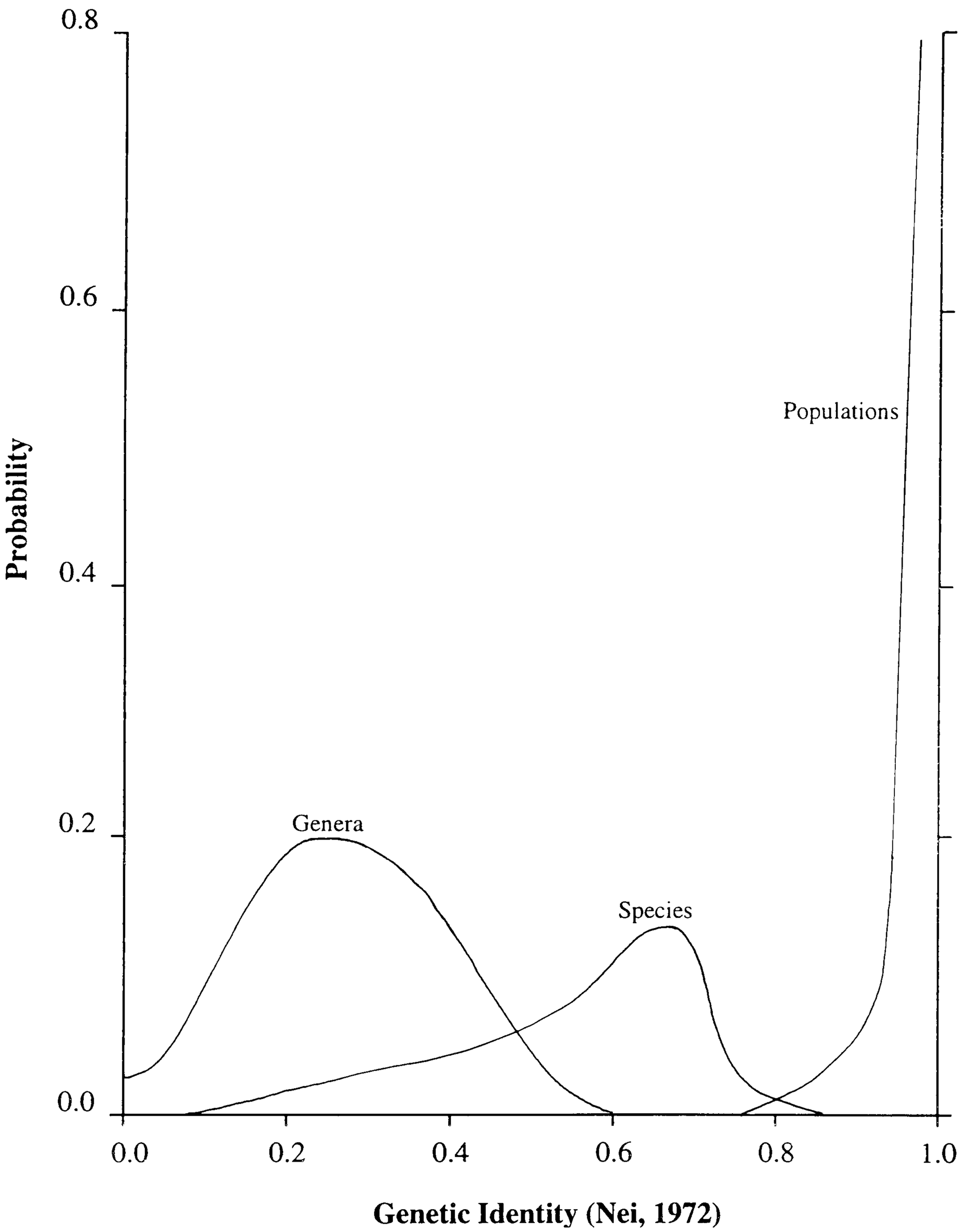


Figure 2.1: Empirically derived frequency distributions for Nei's (1972) genetic identity, *I*, for genetic divergence estimates between conspecific populations, congeneric species and confamilial genera. Redrawn and simplified from Thorpe (1982).

The technique is used widely to settle taxonomic debate in many marine invertebrates (e.g., Greenberg *et al.*, 1996; Röhner *et al.*, 1996) including molluscs (e.g., Côrte-Real *et al.*, 1996; Gardner, 1996) and more specifically cephalopods (e.g., Brierley *et al.*, 1996; Sanjuan *et al.*, 1996). The paucity of octopod genetic data is surprising, given the apparent concern over the unreliability of conventional taxonomic characters in this group.

Methods

Field Sampling

Four sampling programmes yielded benthic octopodids from the study area (Figure 2.2). Between October 1992 and January 1995 fishery observers aboard commercial vessels, which were licensed to fish in the Falkland Islands 200 mile zone using a variety of gears, collected octopodids from the bycatch. Between 4th January and 8th February 1994 the waters around South Georgia and Shag Rocks were surveyed by the Falkland Islands' fishery patrol vessel *MV Cordella* using an FP120 commercial bottom trawl. Between 5th and 28th February 1996 a benthic survey of the coastal shelf of the Weddell Sea was undertaken by the German research vessel *PFS Polarstern*. Five gears (commercial bottom trawl, benthopelagic trawl, Agassiz trawl, box-corer and epibenthic sled) were deployed off Kapp Norvegia and between Vestkapp and Halley Bay. Between 16th November and 26th December 1996 a benthic survey of the Antarctic Peninsula was undertaken by *PFS Polarstern*. Three gears (commercial bottom trawl, benthopelagic trawl and Agassiz trawl) were deployed off Elephant Island, King George Island, and Adelaide Island. Further details of these sampling programmes can be found in Chapter 5.

In addition, and for comparison, *Octopus vulgaris* (Cuvier, 1797) samples were obtained from the Oceanological Observatory at Banyuls-sur-Mer, France in January 1995 whilst *Eledone cirrhosa* (Lamarck, 1798) specimens, caught as a by product in scallop chain dredges, were collected from around the Isle of Man by the Liverpool University research vessel *Roagan* in May 1995.

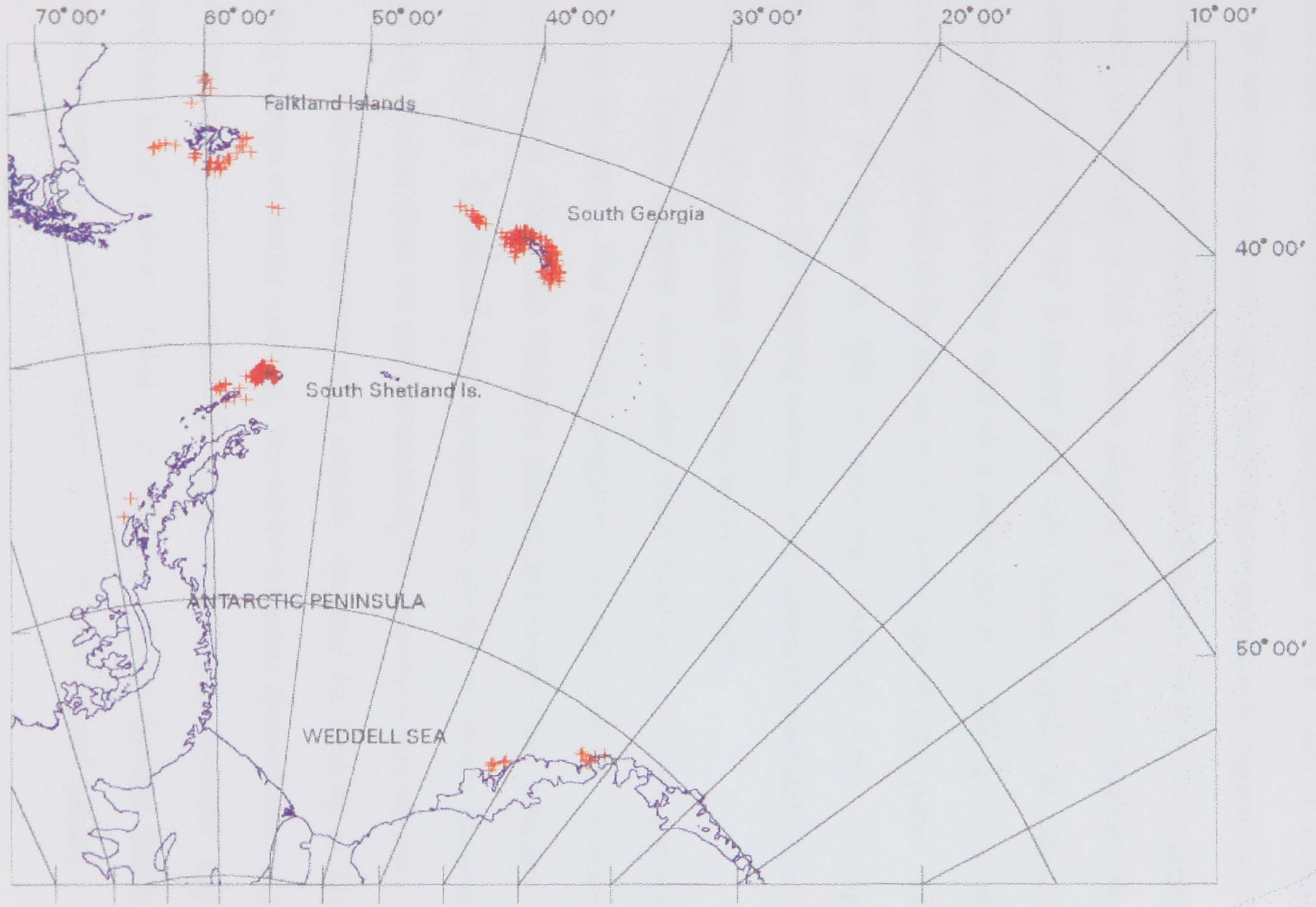


Figure 2.2: Area from which octopodids were collected between October 1992 and December 1996.

All samples were frozen at or below -20°C as soon as possible after capture and stored at this temperature prior to laboratory analysis.

Electrophoresis

Standard procedures for horizontal starch gel electrophoresis have been described by many authors (e.g., Murphy *et al.*, 1990). Gels of dimensions 180 x 150 x 10 mm were prepared using 12.5% hydrolysed starch (Sigma S4501). Samples of mantle tissue were prepared for electrophoresis by homogenising approximately 0.1 g tissue in 100 µl of 1% Triton (Sigma X100). The resulting homogenate was incubated at 4°C for 2 hours and then frozen rapidly in liquid nitrogen. On defrosting, samples were spun in a minifuge at 12,000 g for 4 minutes and the supernatant applied to the gel on 10 x 2 mm strips of filter paper. Gels were run at 4°C for 18 hours at 100 V and the enzymes were subsequently stained histochemically. Gel banding patterns were scored immediately after the completion of staining and genotypes were assigned accordingly.

Tissue samples were taken from thirty-one putative species of octopodid during the study. The species comprised *Octopus vulgaris, Eledone cirrhosa,* five species from around the Falkland Islands and twenty-four Southern Ocean species (Table 2.1). Specimens were assigned to species wherever possible, otherwise to genera. In four cases the generic status of the specimens was unclear.

Fifty-three enzymes were initially screened for activity in *Eledone cirrhosa* using a range of buffer systems. Seventeen enzymes showed activity and eleven loci resolved sufficiently for analysis in species other than *E. cirrhosa*. Eight enzyme loci resolved in all species (Table 2.2) using two buffer systems (Table 2.3). Fewer enzymes were resolved in the Antarctic species than in local species.

Data Analysis

Genotypes and allele frequencies were analysed using the computer programs BIOSYS-1 (Release 1.7) (Swofford and Selander, 1981) and FSTAT (Release 1.2)

Table 2.1: General capture locations of Southern Ocean octopodid species analysed by allozyme electrophoresis.

Species	Falkland Islands	South Georgia	Antarctic Peninsula	Weddell Sea
Octopus tehuelchus	✓			
Benthoctopus eureka	✓			
Enteroctopus megalocyathus	✓			
sp. 7	✓			
sp. 8	✓			
Pareledone turqueti		✓	✓	✓
"*Pareledone*" *polymorpha*	✓	✓	✓	
Thaumeledone gunteri	✓			
Megaleledone senoi			✓	✓
Pareledone sp. 10				✓
Pareledone sp. 11				✓
Pareledone charcoti			✓	✓
Pareledone sp. 13			✓	✓
Pareledone sp. 14			✓	
Pareledone sp. 15			✓	
Pareledone sp. 16			✓	
sp. 17			✓	
"*Pareledone*" sp. 18			✓	
Pareledone sp. 19			✓	
Bentheledone ?albida			✓	
Thaumeledone sp. 21			✓	
Thaumeledone ?brevis			✓	
sp. 22			✓	
Benthoctopus ?levis			✓	✓
Graneledone antarctica			✓	
Graneledone sp. 29			✓	
Graneledone sp. 25			✓	
Pareledone sp. 26			✓	
"*Pareledone*" sp. 28			✓	

Table 2.2: Enzymes screened and resolution obtained. Source of stain recipe is Harris and Hopkinson (1977) except [1]Dando *et al.* (1981), [2]Jeremiah *et al.* (1982), [3]Shaw and Prasad (1970). Details of buffers given in Table 2.3 below.

Enzyme	IUBNC No	Resolved in more than 1 species	Resolved in all species	Buffer
Glycerol 3 phosphate dehydrogenase	1.1.1.8	✓		I
Malate dehydrogenase	1.1.1.37	✓	✓	II
Malate dehydrogenase ($NADP^+$)	1.1.1.40	✓	✓	II
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	✓	✓	II
Phosphogluconate dehydrogenase	1.1.1.44			
Glucose 6 phosphate dehydrogenase	1.1.1.49	✓	✓	II
Octopine dehydrogenase [1]	1.5.1.11	✓		I
Superoxide dismutase	1.15.1.1	✓		I
Purine-nucleoside phosphorylase	2.4.2.1	✓	✓	II
Aspartate amino transferase [2]	2.6.1.1	✓	✓	I
Creatine kinase	2.7.3.2			
Adenylate kinase	2.7.4.3			
Nucleoside triphosphate adenylate kinase	2.7.4.10			
Leucine amino peptidase [3]	3.4.11.1			
Peptidase	3.4.13.*			I
Adenosine deaminase	3.5.4.4	✓	✓	II
Mannose-6-phosphate isomerase	5.3.1.8	✓	✓	II
Glucose-6-phosphate isomerase	5.3.1.9			

Table 2.3: Running conditions of buffers using 12.5% starch gels 10 mm in depth. Recipes from Murphy *et al.*, 1990.

Buffer System		**Voltage** (V/cm)	**Duration** (hours)
I.	**Tris-borate-EDTA II**	11	18
Stock:	0.5 M Tris 0.65 M boric acid 0.02 M disodium EDTA Adjust to pH 8.0		
Electrode:	Undiluted stock solution		
Gel:	1:9 dilution of stock solution		
II.	**Tris-citrate III**	11	18
Stock:	0.75 M Tris 0.25 M citric acid monohydrate Adjust to pH 7.0 with NaOH (pellets)		
Anode:	1:6 dilution of stock solution		
Cathode:	1:7 dilution of stock solution		
Gel:	1:19 dilution of stock solution		

(Goudet, 1994). The fixation index, *f*, (Weir and Cockerham, 1984) was calculated for each population that had polymorphic loci and tested for significant deviation from zero at the 95% confidence level using the normal approximation to the χ^2 distribution (i.e., if $f\sqrt{n} > 1.96$ then H_0 is rejected, where n is the number of individuals in the population sample and the null hypothesis is H_0: $f = 0$). A significant deviation indicates that a population is not in Hardy-Weinberg equilibrium. Genetic identity, *I*, (Nei, 1978) was calculated for pairwise comparisons of samples and clustered using UPGMA (unweighted pair group mean analysis) (Sneath and Sokal, 1973).

Results

Allele frequencies are given for eleven loci (Table 2.4). Of these loci only two exhibited polymorphisms. Polymorphisms were not found in any genus except *Pareledone*. The enzyme locus for isocitrate dehydrogenase (ICD) was polymorphic only in *Pareledone turqueti* specimens from around South Georgia and Shag Rocks, whilst the enzyme locus for glycerol-3-phosphate dehydrogenase (G3PDH) was polymorphic in a range of *Pareledone* species from different locations. Levels of variation are not usually this low in invertebrates, although levels of genetic variation have been shown to be low in other cephalopods (see Brierley *et al.*, 1993b).

The fixation index, *f*, was found to differ significantly from zero in one sample (Table 2.5), that of *Pareledone turqueti* from the Weddell Sea. However, re-analysis of this population using a pooling method (which overcomes some of the problems of using the chi-square test where expected frequencies of some classes are low) (Swofford and Selander, 1981), did not give a significant result ($P = 0.085$). This indicates that there is no significant deviation from Hardy-Weinberg equilibrium in any of the populations investigated.

Although there were differences in allele frequencies between different populations of *P. turqueti* at the polymorphic loci (see Chapter 6) there were no fixed differences in alleles between allopatric populations of any species (Table 2.4).

Table 2.4: Allele frequencies for each putative species at each sample location. (WS) = Weddell Sea, (SG) = South Georgia, (SR) = Shag Rocks, (AP) = Antarctic Peninsula. * indicates lack of activity.

Locus	Allele	*Pareledone turqueti (WS)*	*Pareledone turqueti (AP)*	*Pareledone turqueti (SG)*	*Pareledone turqueti (SR)*	*Pareledone sp. 13 (WS)*	*Pareledone sp. 13 (AP)*	*Pareledone sp. 14*	*Pareledone sp. 27*	*Pareledone sp. 15*	*Pareledone sp. 16*	*Pareledone sp. 19*	*Pareledone sp. 26*	*Pareledone charcoti (WS)*	*Pareledone charcoti (AP)*	*Pareledone sp. 11*	*Pareledone sp. 10*	*sp. 17*	*sp. 22*	*Megaleledone senoi (WS)*	*Megaleledone senoi (AP)*
	(N)	76	3	313	46	40	3	3	1	3	3	3	2	112	3	37	40	3	3	19	3
Aat	A	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0
	B	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Icd	A	0	0	0	0	1	1	0	0	1	0	1	1	1	1	1	1	1	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	C	1	1	0.95	0.18	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	J	0	0	0.05	0.82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mdh	A	0	0	0	0	1	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0
	B	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	1	1	0	0
	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mdhp	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ada	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	E	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	1	1	1	1	1	0	0	0	1	1	0	1	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0
	J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2.4: (cont.) Allele frequencies for each putative species at each sample location. (WS) = Weddell Sea, (SG) = South Georgia, (SR) = Shag Rocks, (AP) = Antarctic Peninsula. * indicates lack of activity.

Locus	Allele	*"Pareledone" sp. 28*	*"Pareledone" polymorpha (SG)*	*"Pareledone" polymorpha (WS)*	*"Pareledone" polymorpha (AP)*	*"Pareledone" sp.18*	*Bentheledone ?albida*	*Thaumeledone ?brevis*	*Thaumeledone sp.21*	*Thaumeledone gunteri*	*Graneledone antarctica*	*Graneledone sp. 25*	*Graneledone sp. 29*	*sp. 7*	*sp. 8*	*Benthoctopus ?levis (WS)*	*Benthoctopus ?levis (AP)*	*Benthoctopus eureka*	*Octopus tehuelchus*	*Octopus vulgaris*	*Enteroctopus megalocyathus*	*Eledone cirrhosa*
	(N)	2	384	84	3	3	3	2	3	2	1	2	3	1	2	2	3	9	40	4	49	6
Aat	A	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	1	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Icd	A	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
	D	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1
	G	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mdh	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	B	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0
	D	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Mdhp	A	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	C	1	1	1	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Ada	A	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	K	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0
	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	O	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Table 2.4: (cont.) Allele frequencies for each putative species at each sample location. (WS) = Weddell Sea, (SG) = South Georgia, (SR) = Shag Rocks, (AP) = Antarctic Peninsula. * indicates lack of activity.

Locus	Allele	*Pareledone turqueti (WS)*	*Pareledone turqueti (AP)*	*Pareledone turqueti (SG)*	*Pareledone turqueti (SR)*	*Pareledone sp. 13 (WS)*	*Pareledone sp. 13 (AP)*	*Pareledone sp. 14*	*Pareledone sp. 27*	*Pareledone sp. 15*	*Pareledone sp. 16*	*Pareledone sp. 19*	*Pareledone sp. 26*	*Pareledone charcoti (WS)*	*Pareledone charcoti (AP)*	*Pareledone sp. 11*	*Pareledone sp. 10*	*Pareledone sp. 17*	*sp. 22*	*Megaleledone senoi (WS)*	*Megaleledone senoi (AP)*
	(N)	76	3	313	46	40	3	3	1	3	3	3	2	112	3	37	40	3	3	19	3
	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1
	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0
Mpi	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Np	E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
G6pdh	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A	0	*	0	0	0	*	*	*	*	*	*	*	1	*	1	1	*	*	0	*
	B	1	*	1	1	1	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*
Sod	C	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*
	D	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*
	E	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	1	*
	A	1	*	1	1	1	*	*	*	*	*	*	*	1	*	1	1	*	*	0	*
	B	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*
	C	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	1	*
Opdh	D	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*
	E	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*
	F	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*
	G	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*
	A	0.42	*	0.94	0.48	0.84	*	*	*	*	*	*	*	0.86	*	0.18	0.90	*	*	0	*
	B	0.22	*	0.01	0	0.14	*	*	*	*	*	*	*	0.05	*	0.21	0.10	*	*	0	*
	C	0	*	0	0	0	*	*	*	*	*	*	*	0.09	*	0.61	0	*	*	0	*
	D	0.36	*	0.05	0.52	0.02	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*
G3pdh	E	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	1	*
	F	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*
	G	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*
	H	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*
	I	0	*	0	0	0	*	*	*	*	*	*	*	0	*	0	0	*	*	0	*

Table 2.4: (cont.) Allele frequencies for each putative species at each sample location. (WS) = Weddell Sea, (SG) = South Georgia, (SR) = Shag Rocks, (AP) = Antarctic Peninsula. * indicates lack of activity.

Locus	Allele	*"Pareledone" sp. 28*	*"Pareledone" polymorpha (SG)*	*"Pareledone" polymorpha (WS)*	*"Pareledone" polymorpha (AP)*	*"Pareledone" sp.18*	*Bentheledone ?albida*	*Thaumeledone ?brevis*	*Thaumeledone sp.21*	*Thaumeledone gunteri*	*Graneledone antarctica*	*Graneledone sp. 25*	*Graneledone sp. 29*	*sp. 7*	*sp. 8*	*Benthoctopus ?levis (WS)*	*Benthoctopus ?levis (AP)*	*Benthoctopus eureka*	*Octopus tehuelchus*	*Octopus vulgaris*	*Enteroctopus megalocyathus*	*Eledone cirrhosa*
	(N)	2	384	84	3	3	3	2	3	2	1	2	3	1	2	2	3	9	40	4	49	6
Mpi	A	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0
	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	F	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Np	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	C	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0
	E	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	H	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	I	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G6pdh	A	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
	D	1	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Sod	A	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	0
	B	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	1	1	1
	C	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	1	1	0	0	0
	D	*	1	1	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	0
	E	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	0
Opdh	A	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	0
	B	*	1	1	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	0
	C	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	0
	D	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	1	0	0	0	0
	E	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	1	0	0	0
	F	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	1	1	0
	G	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	1
G3pdh	A	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	0
	B	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	0
	C	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	0
	D	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	0
	E	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	0
	F	*	1	1	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	0	0
	G	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	1	0	1	0	0
	H	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	1	0	0	0
	I	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	0	1	1

Table 2.5: Tests for departure from Hardy-Weinberg equilibrium where the null hypothesis is Ho: $f = 0$. f, Weir and Cockerham's fixation index; n, number of individuals in the sample; (WS), Weddell Sea; (SR), Shag Rocks; (SG), South Georgia.

Sample	f	$f\sqrt{n}$	Reject Ho?
Pareledone turqueti (WS)	0.400	2.99	Yes
Pareledone turqueti (SG)	0.039	0.69	No
Pareledone turqueti (SR)	0.126	0.85	No
Pareledone charcoti (WS)	0.167	1.56	No
Pareledone sp. 10	-0.094	-0.51	No
Pareledone sp. 11	0.371	1.95	No
Pareledone sp. 13	0.113	0.61	No

Fixed differences in alleles at one or more loci were found between most putative species. *Pareledone* sp. 14 and *Pareledone* sp. 27, however, share the same alleles at all the loci examined (Table 2.4) as do *Pareledone* sp. 15 and *Pareledone charcoti*.

The dendrogram of Nei's (1978) genetic identity (Figure 2.3) was constructed using results from the eight enzyme loci that resolved in all species. Allopatric samples of any one species cluster adjacent to one another. Only in *Pareledone turqueti*, where polymorphic loci were analysed, is there any genetic distance between allopatric populations of a single species. All putative species assigned to the genus *Pareledone* cluster together at the top of the dendrogram. This cluster is in two parts: *Pareledone turqueti* and *P.* sp. 13, 14 and 27 form one cluster, whilst the other *Pareledone* species, together with sp. 22 form the lower cluster. *Graneledone* sp. 25 and 29, cluster together but away from the established species *Graneledone antarctica*. The latter clusters with the other deep water species encountered in the study. The five species with a biserial sucker arrangement cluster together.

Discussion

The levels of polymorphism encountered in this study were low in comparison to levels seen in other marine invertebrates (see Nevo *et al.*, 1984). Selander (1976) gives 0.58 as an average value for the proportion of polymorphic loci in published work on invertebrates. Many studies on squid, however, reveal similar levels of polymorphisms to those found here (summarised by Brierley *et al.*, 1993b) as does a recent study on *Sepia* (Sanjuan *et al.*, 1996), and it appears that lack of variability (at least as realised by allozymes) is a phenomenon common to the class Cephalopoda; this is discussed in greater detail in Chapter 6. It could be argued that fewer polymorphisms were revealed in this study due to the small sizes of some of the samples, however, ten of the samples contained forty or more specimens, allowing ample opportunity for polymorphisms to be revealed.

Non of the samples displaying polymorphisms deviated significantly from Hardy-Weinberg equilibrium. Factors commonly associated with such deviations

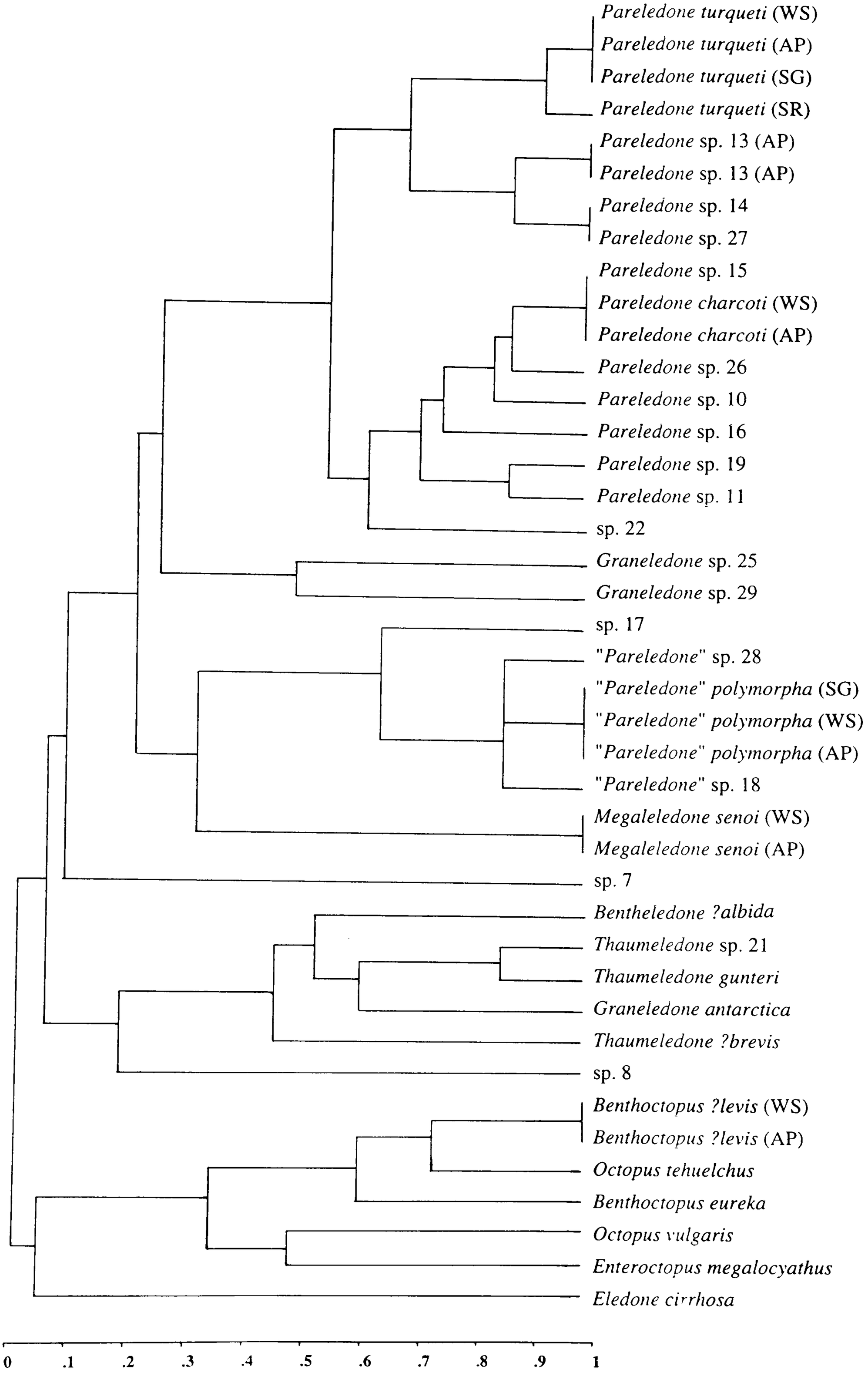


Figure 2.3: UPGMA dendrogram of Nei's (1978) genetic identity based on eight enzyme loci. (WS), Weddell Sea; (AP), Antarctic Peninsula; (SG), South Georgia; (SR), Shag Rocks.

include inbreeding, assortative mating and population subdivision and hence cryptic speciation. Cryptic speciation is a common phenomenon in marine invertebrate taxa and many previously unrecognised species have been identified using electrophoresis, including molluscan species (e.g., Murphy, 1978; Mastro *et al.*, 1982) and more specifically cephalopods (e.g., Smith *et al.*, 1987; Brierley *et al.*, 1993b). There is however no evidence of the presence of cryptic species in any of the samples collected for this study.

The lack of polymorphisms aids the intuitive assumption that differences in alleles between populations are fixed even when the results suggesting this are from very small samples. The power of electrophoretic analysis is high, however, regardless of the level of polymorphism. Consider a theoretical case where fixed allele differences have been found at a locus using only three individuals of each operational taxonomic unit (OTU), i.e., all three specimens of OTU 1 are homozygous for allele 1, whilst all three specimens of OTU 2 are homozygous for allele 2. Since allele 2 is not present in any of the three specimens (and therefore six alleles) of OTU 1 its empirical frequency in this morph is less than 1 in 6. If OTU 2 is from the same gene pool, then the chance that an individual from this OTU is homozygous for allele 2 is $P < (1/6)^2$, and the chance that all three individuals from OTU 2 are homozygous for this allele is $P < [(1/6)^2]^3$ or $P < 2.1 \times 10^{-5}$. Hence fixed allele differences are highly significant even when found between samples of only three individuals.

There were no fixed differences in alleles between allopatric samples thought to be conspecific. Had there been, this would simply have indicated that both samples were not drawn from the same gene pool, and not that they were necessarily separate species. The applicability of specific status would have to be assessed on the genetic distance between samples with reference to Thorpe's (1982) suggested critical value of $I = 0.85$.

Between sympatric samples the situation is slightly different. Since sympatric populations that do no exchange genes are considered different species under any of

the current species concepts, allozymes are "at their most powerful in discriminating and identifying sympatric species" (Thorpe and Solé-Cava, 1994). These authors suggest that *I* values are of limited relevance to taxonomic problems compared with gene frequency data in this situation and, if gene pools are sympatric, they recommend that fixed differences in alleles should not be disregarded even if *I* values are unusually high (e.g., Avise and Ayala, 1976; Solé-Cava *et al.*, 1985). Hence even though $I = 0.875$ (i.e., above the critical value of $I = 0.85$) for several closely related sympatric putative species (Figure 2.3), specific status is justified on the basis of fixed differences in alleles at diagnostic loci (Table 2.4). Of course, not all the putative species were sympatric but all allopatric species had a genetic identity value of less than 0.85 and their specific status is therefore also justified.

No fixed differences in alleles were found between two pairs of species. Allozymes can only conclude that no significant differences could be found between species pairs and cannot prove that two samples are conspecific. It is always a possibility that differentiation may be present at loci which have not been examined and only a low proportion of variation in alleles may be detectable by standard electrophoresis (Lewontin, 1974; Nei, 1987). However, in the absence of electrophoretic confirmation there needs to be other conclusive evidence to support specific status. Only one specimen of *Pareledone* sp. 27 was captured, and although similar to *Pareledone* sp. 14 it was separated from the latter on the basis of external colour patterning. Octopodids, however, are renowned for their ability to change colour (e.g., Norman, 1991) and this alone is insufficient evidence for specific status. It also appeared that the ink sac was either very reduced or possibly absent in this specimen, but with no other examples to examine, and all other features suggesting this specimen belongs in the genus *Pareledone,* this specimen must, at present, be considered conspecific with *Pareledone* sp. 14. Forty-two specimens of *Pareledone* sp. 15 were caught at seven stations on the Antarctic Peninsula (see Chapter 5). Although this species could not be separated from *Pareledone charcoti* by electrophoresis, it differed consistently from *P. charcoti* in colour, shape, and the

arrangement of papillae and was caught in sufficient numbers such that detailed morphometric measurements could be taken from a range of specimens. When twenty-four indices and counts were analysed (see Chapter 3, Figure 3.8) three other species clustered more closely to *Pareledone charcoti* than did *Pareledone* sp. 15. The discriminant function analysis (see Chapter 3, Table 3.12) did not erroneously reclassify any specimens of *P.* sp. 15 as *P. charcoti*, nor did it reclassify any specimens of *P. charcoti* as *P.* sp. 15. It is therefore likely that the specific status of *Pareledone* sp. 15 is justified.

Figure 2.3 reveals that all the new and recognised species of *Pareledone* group into two closely related clusters. The first comprises only species of *Pareledone* that have smooth skin, whilst the second comprises *Pareledone* species that have some form of skin papillation. Although there is great variation in the types of papillation seen in *Pareledone* species (see Chapter 4), it is possible that these are all derived from a common ancestor. The cluster of papillose species also includes sp. 22 whose generic status was not clarified prior to electrophoresis. The morphology of sp. 22 and sp. 17 was very similar (see also Chapter 3) and it was thought that they probably belonged in the same genus, although it was unclear which genus this should be. Their inclusion in *Pareledone* was doubted because of the relative depth of their web which was much greater than that seen in other *Pareledone* species. Sp. 17, however, has clustered out separately from sp. 22 and is most closely associated with "*Pareledone*" *polymorpha* and "*Pareledone*" sp. 18. However, regardless of the status of sp. 22 and sp. 17, it appears that the genus *Pareledone* is valid and that all the new species tentatively assigned to *Pareledone* do belong within this genus.

"*Pareledone*" *polymorpha* and similar new species cluster some distance away lending support to previous suggestions that "*P.*" *polymorpha* should be removed from the genus *Pareledone* (see Chapter 1). *Megaleledone senoi* clusters most closely with "*Pareledone*" *polymorpha* rather than with the large *Pareledone*

grouping, refuting the suggestions of Voss (1988b) who refers to the species as *Pareledone senoi* (see Chapter 1).

Also of interest is that all the deep water species (sp. 8, *Bentheledone ?albida*, *Thaumeledone ?brevis, Thaumeledone* sp. 21, *Thaumeledone gunteri, Graneledone antarctica*) all group together and separate from the other new species tentatively placed in *Graneledone* on the bases of their granular skin and lack of ink sac. It is quite possible that the latter have been misplaced and to reflect this uncertainty they will henceforward be referred to as *?Graneledone* sp. 25 and *?Graneledone* sp. 29. In the dendrogram, groups of species with an ink sac (see Chapter 1 for diagnoses of genera) are interspersed with groups of species without ink sacs. This perhaps casts doubt on the validity of using presence / absence of ink sac as a subfamiliar character.

The five species with double sucker rows (*Benthoctopus, Octopus* and *Enteroctopus*) all group together, as might be expected under traditional systematic schemes (see Chapter 1). Interestingly *Eledone cirrhosa* (which has a single row of suckers) clusters more closely with these species than with those which also have uniserial suckers. This perhaps supports suggestions that its single sucker row is a secondarily derived character; however, *Eledone* is not closely aligned with either group.

Furthermore, care must be taken when using electrophoretic data to draw inferences about higher taxa, especially from data based on eight loci. Although the technique is extremely useful at the specific level, there is no critical value of *I* that clearly delineates between higher taxonomic levels. In part this is probably because there are no biological criteria for defining the higher taxa, and genera defined by genetic identity levels are likely to be of little use. Whether it is even of value for genera to encompass similar amounts of genetic diversity is a matter for taxonomic debate (see Van Valen, 1973). In addition, though, unless a very large number of loci are resolved for all the species under consideration, phylogenetic resolution at higher taxonomic levels is poor. Genetic distance estimates are far more severely affected

by the number of loci sampled than by the number of individuals sampled (Gormon and Renzi, 1979; Nei, 1978) and there is an "unfortunate tendency for electrophoretic data to be subjected to far more exact analysis than is justified" (Thorpe and Solé-Cava, 1994). Most genetic divergence estimates based on practicable numbers of loci will have large errors, and systematic distinctions are made using differences smaller than these errors (Thorpe and Solé-Cava, 1994).

Activity in some of the samples used in this study was poor. Tissue samples from the Antarctic Peninsula (which showed activity for the fewest loci) were transported to the UK in a thermally insulated box, rather than in a freezer aboard a ship, and it is likely that this is the cause of some loss of activity. It is likely that the extended periods of frozen storage, to which all the tissue samples in this study were subjected (except for *Eledone cirrhosa*), played a role in reducing enzyme activity further. This phenomenon is well documented in squid (e.g., Carvalho and Loney, 1989; Brierley, 1992). However, even if more loci had been available, higher level taxonomic problems would still have been better addressed using molecular methods. Furthermore, the primary goal of clarifying the status of existing and new *Pareledone* species has been achieved.

Chapter 3

Taxonomic studies on Octopodidae from the Southern Ocean with special reference to the genus *Pareledone*: a phenetic perspective.

Introduction

Recognition of discrete taxa is a problem in groups, such as the Octopodidae, that display a great degree of homeomorphy at low taxonomic levels (Hageman, 1991). This problem is exacerbated in the Octopodidae because of the lack of hard parts and the phenotypic plasticity of the soft parts (see Chapter 1). Because of the soft nature of octopuses, the fossil record is poor and hence, there is often uncertainty as to which character states are primitive or derived and classifications are necessarily based on characters that have not been validated.

The characters relied upon to define cephalopod taxa have rarely been tested for systematic reliability (Voss, 1977). Many are based on simple morphometric indices (Roper and Voss, 1983) but these have been criticised because of the susceptibility of morphometric characters to environmental variation (Avise, 1994). Although the indices of Roper and Voss (1983) are commonly used in taxonomic descriptions and redescriptions, their usefulness is unconfirmed; in cases where taxonomic status is controversial it is far more common for researchers to resort to biochemical and molecular methods (e.g., Bonnaud *et al.*, 1994; Boucher-Rodoni *et al.*, 1995; Nishigushi *et al.*, 1995; Perez-Losada *et al.*, 1995; Brierley *et al.*, 1996; Sanjuan *et al.*, 1996) than to test the discriminatory powers of the indices recorded. Morphometric measurements have, however, been used, in conjunction with multivariate analyses, to separate two populations of the squid *Loligo forbesi* (Pierce *et al.*, 1994a), although these researchers suggested that morphometric measurements are not usually sufficiently sensitive at the population level (Pierce *et al.*, 1994b).

Daly and Rodhouse (1994) undertook a study of comparative morphology of two sympatric *Pareledone* species from South Georgia. This showed that several morphometric indices, including indices derived from beak morphology, could be used to discriminate between the two species. However, recent work on one species ("*Pareledone*" *polymorpha*) involved in the study suggests that this might not belong to the genus *Pareledone* (see Chapters 1 and 2). The study may therefore only

confirm that the indices used are useful in discriminating between genera and not between species.

The use of beak morphology in cephalopod taxonomy was developed by Clarke (1962, 1980, 1986). In squid it has proved possible to identify species from beaks found in the stomachs of vertebrate predators (Clarke, 1996; Croxall and Prince, 1996) and it has been concluded that discrimination is good at the specific level (Wolff and Wormuth, 1979). It is generally accepted that there is a greater degree of homeomorphy in octopus beaks and Voss (1977) considers beak morphology to be unreliable in octopus taxonomy. Although descriptions of beak morphology are often not included in taxonomic descriptions (e.g., Kubodera and Okutani, 1994), they are, however, recommended by Roper and Voss (1983).

This study was designed to address some of the uncertainty surrounding the use of morphometric measurements in the Octopodidae by investigating

a) whether discrete taxa can be recognised using morphometric characters

b) which types of character are most useful for making taxonomic distinctions

c) how many characters are required to make taxonomic distinctions

d) whether the same types of characters can be used to make taxonomic distinctions at different taxonomic levels and

e) whether the distinctions made reflect those suggested by the biochemical genetic analyses in Chapter 2 and whether they are of phylogenetic use.

Methods

Octopuses were sampled from around the Falkland Islands and from three areas in the Southern Ocean (see Chapter 2). Specimens were assigned to species wherever possible, otherwise to genera. Twenty-four counts and indices (see Roper and Voss, 1983) were calculated (Table 3.1) from 454 freshly killed specimens comprising fifteen species (Table 3.2). The Box-Cox transformation (Box and Cox, 1964) was utilised to transform indices to normality whilst counts were analysed non-parametrically.

Table 3.1: Description of twenty-four counts and indices utilised in discriminant analysis. * indicates characters specific to males. (Diagrammatic representations of many measurements may be found in Roper and Voss, 1983).

Index / Count	Abbr.	Description
Mantle Width Index	MWI	mantle width / dorsal mantle length (ML) x 100
Head Width Index	HWI	head width / ML x 100
Eye Diameter Index	EDI	diameter of eye across bulbus / ML x 100
Eye Orifice Index	EOI	diameter of the opening of the eye / ML x 100
Pallial Aperture Index	PAI	pallial aperture / ML x 100
Funnel Length Index	FuLI	funnel length from anterior opening to posterior border along ventral midline / ML x 100
Free Funnel Index	FFuI	funnel length from anterior opening to point of dorsal attachment / ML x 100
Web Depth Index	WDI	length from mouth to midpoint of deepest sector between arms / longest arm length x 100
Mantle Arm Index	MAI	ML / longest arm length x 100
Arm Width Index	AWI	width of stoutest arm / ML x 100
Arm Length Index L1	L1	length of first left arm / ML x 100
Arm Length Index L2	L2	length of second left arm / ML x 100
Arm Length Index L3	L3	length of third left arm / ML x 100
Arm Length Index L4	L4	length of fourth left arm / ML x 100
Arm Length Index R1	R1	length of first right arm / ML x 100
Arm Length Index R2	R2	length of second right arm / ML x 100
Arm Length Index R3	R3*	length of third right arm / ML x 100
Arm Length Index R4	R4	length of fourth right arm / ML x 100
Opposite Arm Index	OAI*	length of third right arm / third left arm x 100
Arm Sucker Index	ASI	diameter of largest normal arm sucker / ML x 100
Ligula Length Index	LLI*	length of ligula / total length of third arm x 100
Hectocotylised Sucker Count	HSC*	number of suckers on the hectocotylised arm
Highest Number of Suckers	HNS	highest number of suckers found on any arm
Outer Gill Lamellae Count	OGL	number of outer gill lamellae

Table 3.2: Number of specimens of each species used to test the discriminant power of twenty-four morphometric indices and counts.

Species	Total Number	Number of Males
Pareledone turqueti	43	15
"*Pareledone*" *polymorpha*	34	21
Megaleledone senoi	28	20
Pareledone sp. 10	25	18
Pareledone sp. 11	19	10
Pareledone charcoti	83	48
Pareledone sp. 13	24	12
Pareledone sp. 14	20	11
Pareledone sp. 15	17	15
Pareledone sp. 16	36	18
sp. 17	35	18
"*Pareledone*" sp. 18	23	9
Pareledone sp. 19	36	17
sp. 22	11	5
?Graneledone sp. 29	20	8

Table 3.3: Number of specimens of each species used to test the discriminant power of indices derived from beak morphometrics.

Species	Total Number
Pareledone turqueti	69
"*Pareledone*" *polymorpha*	20
Benthoctopus eureka	9
Octopus tehuelchus	17
Megaleledone senoi	9
Pareledone sp. 10	29
Pareledone sp. 11	21
Pareledone charcoti	54
Pareledone sp. 13	30

Initially eighteen indices were analysed in all specimens. Twenty-one indices (three of which involved characters specific to males) were then analysed in the 245 males in the sample. Canonical discriminant analysis (CDA) (SAS, Release 6.11) was used to produce a discriminant function in order to test the performance of morphometric measurements in discriminating between species. CDA produces a linear combination of variables that have the greatest among-group variation relative to their within-group structure (Digby and Kempton, 1987).

Mahalanobis distances (the Euclidean distance between the n-dimensional means of the canonical variables) were calculated for each pairwise species comparison and clustered using unweighted pair group mean analysis (UPGMA) (Sneath and Sokal, 1973). For comparison, genetic identity, *I*, (Nei, 1978) was calculated (from the allele frequency matrix in Chapter 2, Table 2.4) for the same pairwise comparisons and clustered similarly. Stepwise discriminant function analysis, a procedure that selects a subset of discriminant variables according to the significance level of an analysis of covariance, was employed on all twenty-one indices to investigate whether any should be excluded from the analysis.

The counts were analysed using non-parametric (*k*-nearest neighbour, with $k = 3$) discriminant function analysis (SAS, Release 6.11). Pairwise squared distances calculated between species were compared to genetic distances. Finally all twenty-four counts and indices were analysed together using this non-parametric method.

A separate data set was used to test the usefulness of beak morphology in identifying species. Beaks were dissected from 258 specimens comprising 9 species (Table 3.3), cleaned, and stored in ethanol. Measurements were made using image analysis software (NIH Image 1.58 VDM). Seven dimensions (adapted from Clarke, 1986) were recorded (Figure 3.1) and seven indices calculated (Table 3.4). The Box-Cox transformation was utilised to normalise indices and the resulting data were analysed parametrically as described above.

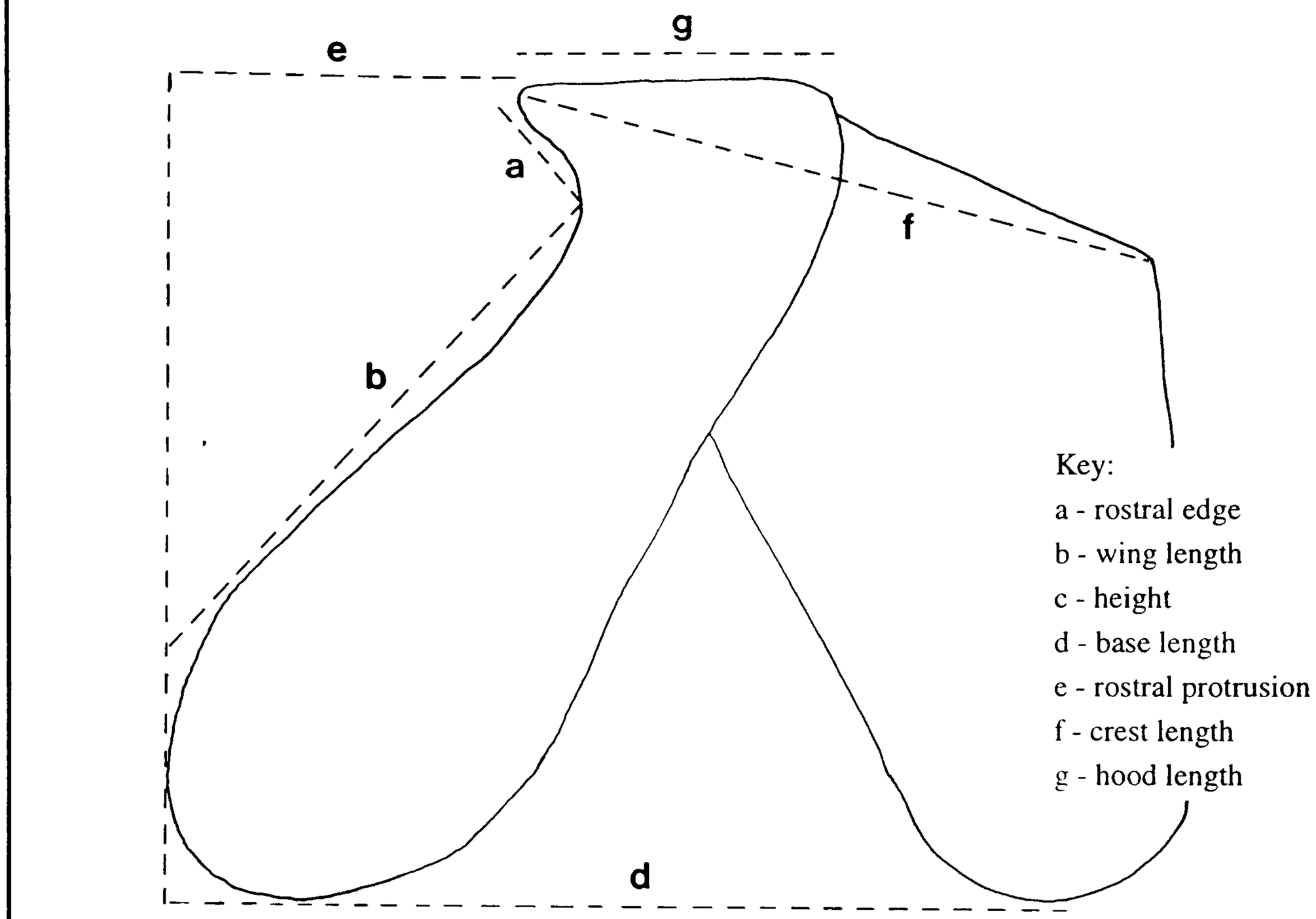


Figure 3.1: Diagrammatic representation of an octopus beak illustrating the seven dimensions measured in this study (adapted from Clarke, 1986).

Table 3.4: Description of seven indices derived from morphometric measurements of beaks. (See also Figure 3.1 above.)

Index	Abbr.	Description
Wing Length Index	WLI	wing length / rostral edge
Height Index	HI	height / base length
Rostral Protrusion Index	RPI	base length / rostral protrusion
Hood Length Index	HLI	crest length / hood length
Crest Length Index	CLI	base length / crest length
Rostral Edge Index	REI	rostral edge / dorsal mantle length
Base Length Index	BLI	base length / dorsal mantle length

Results

Analysis of eighteen indices in male and female specimens of fifteen species of octopus

Canonical discriminant analysis of eighteen indices derived from morphometric measurements produced fourteen canonical variates; the first three canonical variates accounted for 75% of the total variance (Table 3.5). Character loadings (canonical coefficients; Table 3.6) describe the relative contribution of each index to the discriminant function. Web depth index (WDI) makes a large contribution to the first canonical co-efficient (accounting for 38% of the variance). Funnel length index (FuLI) and mantle arm index (MAI) make the largest contributions to the second canonical co-efficient (accounting for 27% for the variance). The discriminant function reclassified 68% of the specimens correctly (Table 3.7). In the phenogram of Mahalanobis distances only *Megaleledone senoi* clusters separately (Figure 3.2). *Pareledone* sp. 16 clusters with sp. 17 and sp. 22 away from all the other *Pareledone*. *?Graneledone* sp. 29 clusters most closely with "*Pareledone*" *polymorpha* and "*Pareledone*" sp. 18.

Analysis of twenty-one indices in male specimens only of fifteen species of octopus

Canonical discriminant analysis produced fourteen canonical variates; the first three canonical variates accounted for 71% of the total variance (Table 3.8). Web depth index (WDI) makes a large contribution to the first canonical co-efficient (accounting for 38% of the variance) as does arm length index R3 (Table 3.9). WDI, arm length indices R3 and L3, and opposite arm index (OAI) make the largest contributions to the second canonical co-efficient (accounting for 22% of the variance). A plot of the first two canonical variates (Figure 3.3) shows that only *Megaleledone senoi* separates out clearly. Removing *Megaleledone senoi* and the *Pareledone* with papillose skin (shown in blue) from this plot (Figure 3.4) reveals greater separation between the other groups. The smooth skinned *Pareledone* (shown in red) do not show good separation at the species level. This is more evident

Table 3.5: Analysis of eighteen indices in male and female specimens of fifteen species of octopus. Eigenvalues of the correlation matrix calculated by canonical correlation.

Canonical Variate	Eigenvalue	Proportion of Variance	Cumulative Proportion
1	2.7815	0.3773	0.3773
2	1.9542	0.2651	0.6423
3	0.8273	0.1122	0.7545
4	0.6024	0.0817	0.8362
5	0.4884	0.0662	0.9025
6	0.2192	0.0297	0.9322
7	0.1667	0.0226	0.9548
8	0.1134	0.0154	0.9702
9	0.0783	0.0106	0.9808
10	0.0562	0.0076	0.9885
11	0.0464	0.0063	0.9947
12	0.0225	0.0031	0.9978
13	0.0102	0.0014	0.9992
14	0.0061	0.0008	1.0000

Table 3.6: Analysis of eighteen indices in male and female specimens of fifteen species of octopus. Total-sample standardized canonical coefficients.

Index	Coefficient 1	Coefficient 2	Coefficient 3
MWI	0.143	-0.163	0.552
HWI	0.318	0.479	0.410
EDI	-0.187	0.537	0.144
EOI	0.402	0.210	-0.055
PAI	-0.147	0.172	-0.654
FuLI	0.251	0.691	0.302
FFuI	0.204	-0.145	0.376
WDI	1.782	-0.468	-0.204
MAI	-0.688	0.676	-0.064
AWI	-0.035	0.134	-0.155
L1	-0.109	0.064	0.600
L2	0.098	-0.027	-0.100
L3	0.092	-0.276	-0.684
L4	-0.384	-0.267	0.210
R1	0.060	0.019	1.150
R2	0.249	-0.188	-0.326
R4	0.532	-0.185	0.128
ASI	-0.190	-0.264	-0.807

Table 3.7: Analysis of eighteen indices in male and female specimens of fifteen species of octopus. Resubstitution summary using linear discriminant function. Number and percent of observations classified into each species.

Species	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	Error
(1) *Pareledone turqueti*	31	.	.	2	.	2	5	2	1	.	.	.	.	.	.	12
	72.1	.	.	4.7	.	4.7	11.6	4.7	2.3	.	.	.	.	.	.	0.28
(2) "*Pareledone*" *polymorpha*	.	17	1	.	.	5	3	.	.	1	.	3	3	.	1	17
	.	50.0	2.9	.	.	14.7	8.8	.	.	2.9	.	8.8	8.8	.	2.9	0.50
(3) *Megaleledone senoi*	.	.	26	.	.	.	.	.	1	.	1	.	.	.	.	2
	.	.	92.9	.	.	.	.	.	3.6	.	3.6	.	.	.	.	0.07
(4) *Pareledone* sp. 10	6	2	.	10	.	2	.	3	.	.	.	.	2	.	.	15
	24.0	8.0	.	40.0	.	8.0	.	12.0	.	.	.	.	8.0	.	.	0.60
(5) *Pareledone* sp. 11	.	1	.	2	9	7	.	.	.	.	.	.	.	.	.	10
	.	5.3	.	10.5	47.4	36.8	.	.	.	.	.	.	.	.	.	0.53
(6) *Pareledone charcoti*	1	4	1	2	8	45	.	.	1	7	7	.	5	.	2	37
	1.2	4.8	1.2	2.4	9.6	54.2	.	.	1.2	8.4	8.4	.	6.0	.	2.4	0.46
(7) *Pareledone* sp. 13	3	1	.	.	.	1	19	.	.	.	.	.	.	.	.	5
	13.0	4.2	.	.	.	4.2	79.2	.	.	.	.	.	.	.	.	0.21
(8) *Pareledone* sp. 14	1	.	.	1	.	1	.	17	.	.	.	.	.	.	.	3
	5.0	.	.	5.0	.	5.0	.	85.0	.	.	.	.	.	.	.	0.15
(9) *Pareledone* sp. 15	.	.	.	.	.	5	.	1	9	1	.	.	1	.	.	8
	.	.	.	.	.	29.4	.	5.9	52.9	5.9	.	.	5.9	.	.	0.47
(10) *Pareledone* sp. 16	.	.	.	.	.	1	.	.	.	33	.	1	1	.	.	3
	.	.	.	.	.	2.8	.	.	.	91.7	.	2.8	2.8	.	.	0.08
(11) sp. 17	.	.	.	.	.	1	.	.	1	.	25	.	2	4	2	10
	.	.	.	.	.	2.9	.	.	2.9	.	71.4	.	5.7	11.4	5.7	0.29
(12) "*Pareledone*" sp. 18	.	4	.	.	.	.	.	.	1	.	.	17	.	.	1	6
	.	17.4	.	.	.	.	.	.	4.3	.	.	73.9	.	.	4.3	0.26
(13) *Pareledone* sp. 19	.	.	.	.	.	9	.	.	.	2	.	.	25	.	.	11
	.	.	.	.	.	25.0	.	.	.	5.6	.	.	69.4	.	.	0.31
(14) sp. 22	.	.	.	.	.	.	.	.	.	.	6	.	.	5	.	
	.	.	.	.	.	.	.	.	.	.	54.5	.	.	45.5	.	0.55
(15) ?*Graneledone* sp. 29	.	.	.	.	.	1	.	.	.	.	.	1	.	.	18	2
	.	.	.	.	.	5.0	.	.	.	.	.	5.0	.	.	90.0	0.10
													Overall Error Rate			0.32

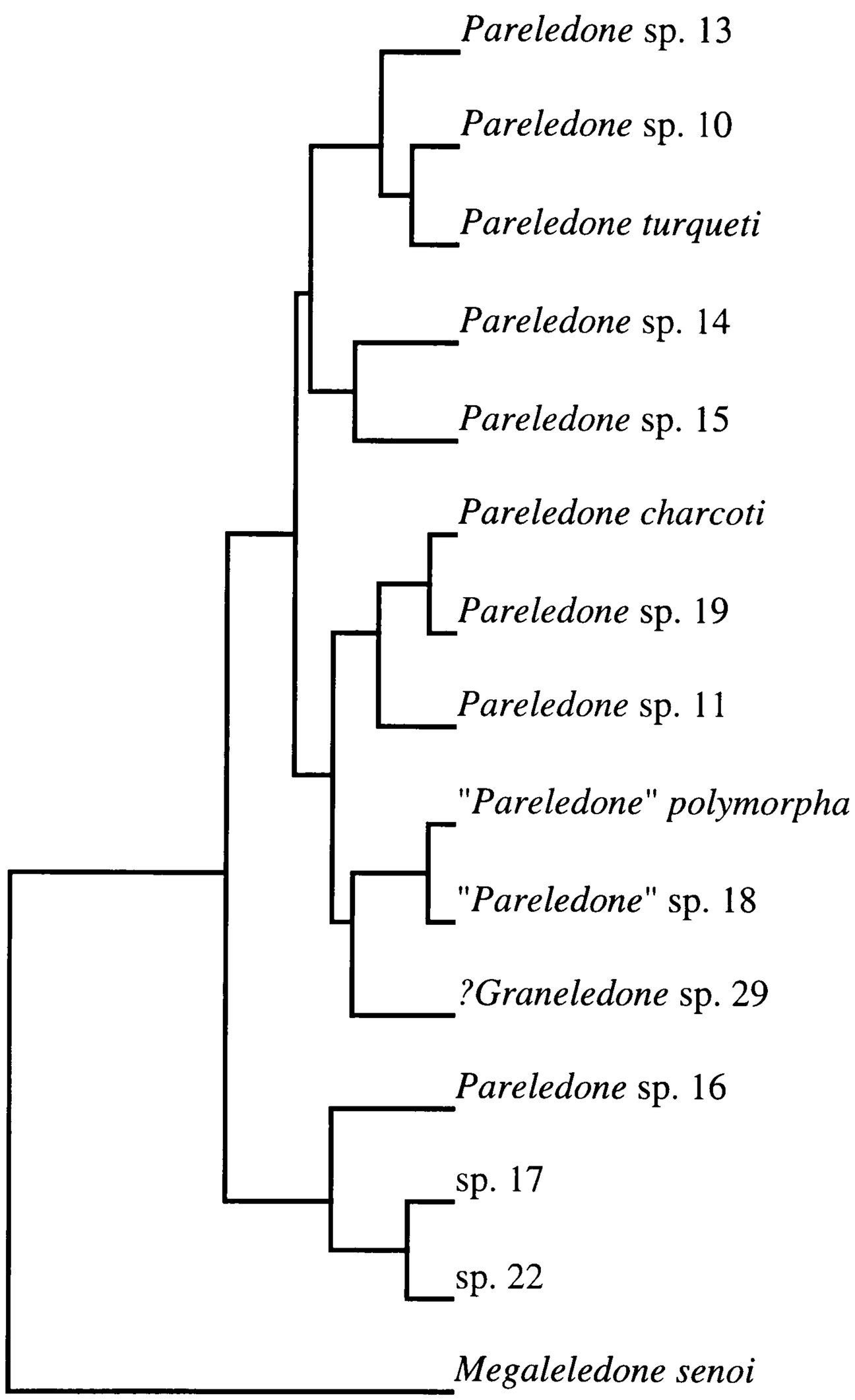


Figure 3.2: Analysis of eighteen indices in male and female specimens of fifteen species of octopus. Pairwise squared distances between species clustered using UPGMA.

Table 3.8: Analysis of twenty-one indices in male specimens only of fifteen species of octopus. Eigenvalues of the correlation matrix calculated by canonical correlation.

Canonical Variate	Eigenvalue	Proportion of Variance	Cumulative Proportion
1	4.4398	0.3790	0.3790
2	2.5662	0.2191	0.5980
3	1.2706	0.1085	0.7065
4	0.9689	0.0827	0.7892
5	0.7884	0.0673	0.8565
6	0.5583	0.0477	0.9042
7	0.3527	0.0301	0.9343
8	0.2178	0.0186	0.9529
9	0.1802	0.0154	0.9682
10	0.1399	0.0119	0.9802
11	0.0956	0.0082	0.9884
12	0.0751	0.0064	0.9948
13	0.0544	0.0046	0.9994
14	0.0069	0.0006	1.0000

Table 3.9: Analysis of twenty-one indices in male specimens only of fifteen species of octopus. Total-sample standardized canonical coefficients.

Index	Coefficient 1	Coefficient 2	Coefficient 3
MWI	0.323	0.080	-0.581
HWI	0.094	0.127	-0.153
EDI	-0.475	-0.168	-0.218
EOI	-0.087	0.374	0.012
PAI	0.092	-0.124	0.619
FuLI	-0.529	0.294	-0.333
FFuI	0.147	0.256	-0.360
WDI	1.019	1.436	-0.174
MAI	-0.848	0.215	0.352
AWI	-0.229	0.238	-0.165
L1	-0.216	-0.257	-0.079
L2	0.263	0.425	0.352
L3	-0.771	2.424	-5.672
L4	0.137	-0.599	0.335
R1	-0.129	0.162	-1.367
R2	0.220	0.355	0.007
R3	0.926	-2.213	6.572
R4	0.504	0.056	0.103
OAI	-0.586	1.387	-3.212
ASI	0.355	-0.334	0.707
HLI	-0.784	0.556	0.575

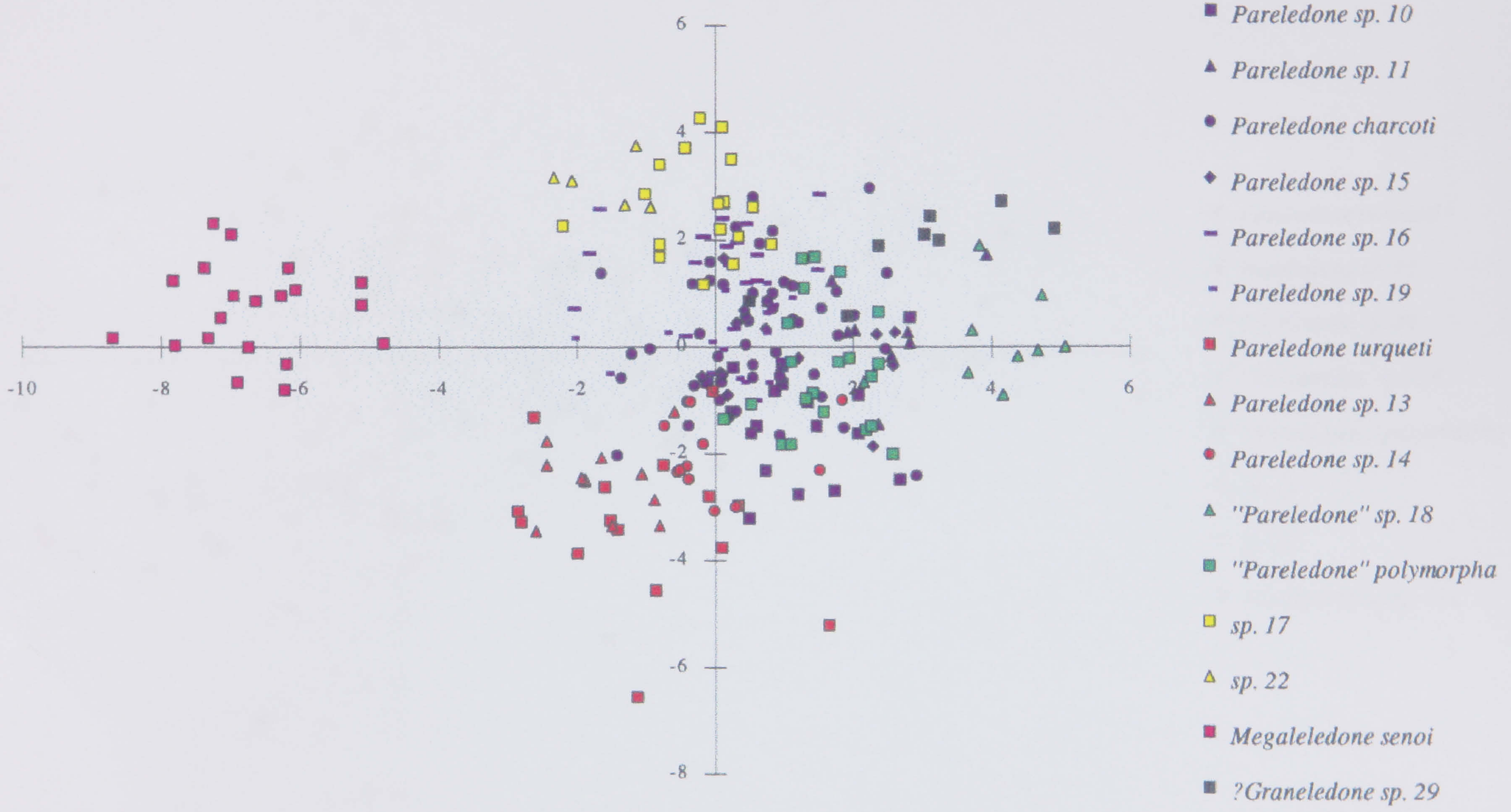


Figure 3.3: **Analysis of twenty-one indices in male specimens only of fifteen species of octopus. Plot of canonical variable 1 against canonical variable 2.**

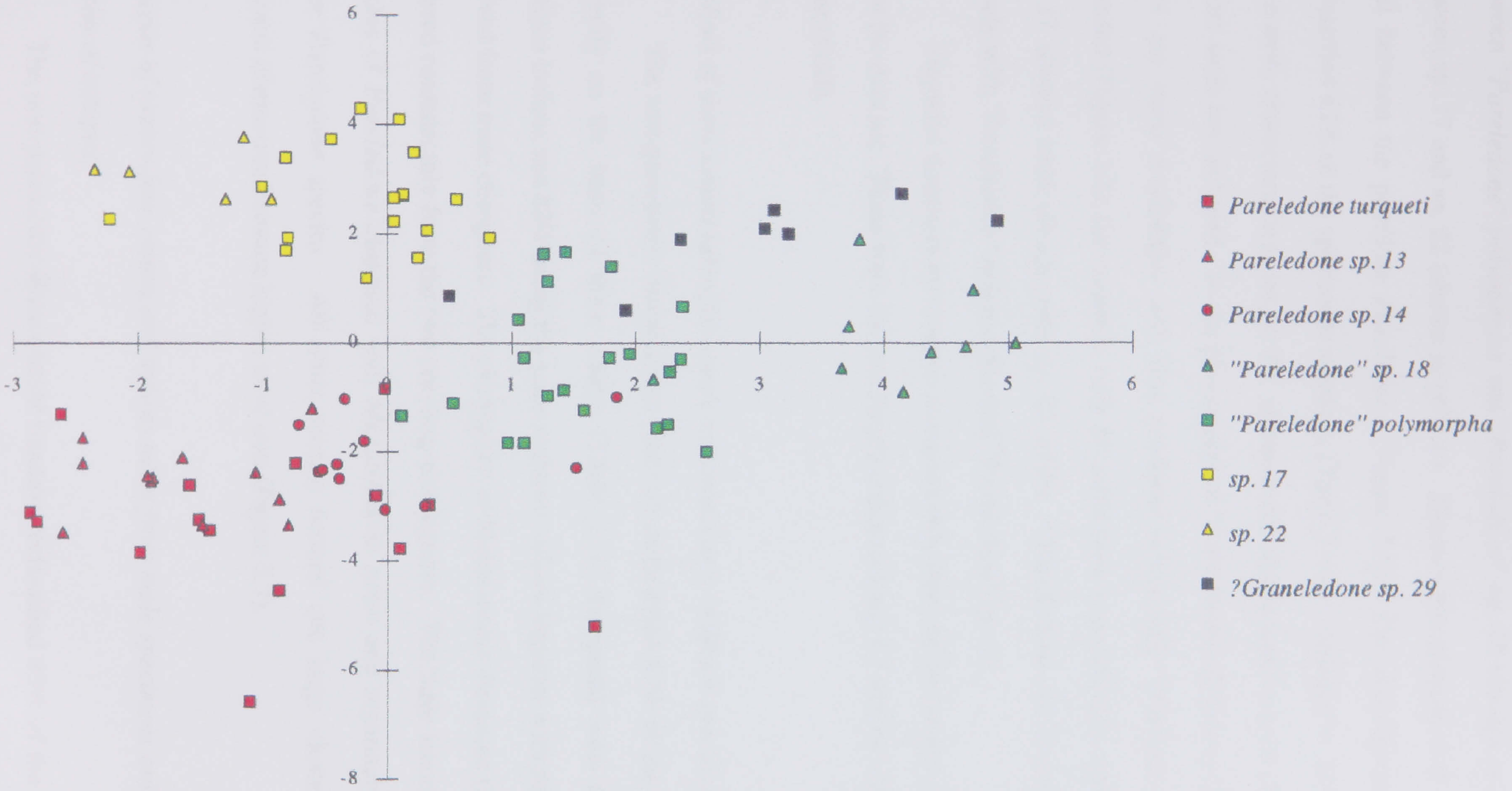


Figure 3.4: Analysis of twenty-one indices in male specimens only of fifteen species of octopus. Plot of canonical variable 1 against canonical variable 2. For clarity, ***Megaleledone senoi*** and ***Pareledone*** species with papillose skin have been excluded.

between "*Pareledone*" *polymorpha* and "*Pareledone*" sp. 18 (shown in green) and between sp. 17 and sp. 22 (shown in yellow). There is less separation at the species level between the papillose *Pareledone* (Figure 3.5). The discriminant function reclassified 82% of the specimens correctly (Table 3.10), compared to 68% when the three male characters (arm length R3, opposite arm index and hectocotylised length index) were not included. In the phenogram of Mahalanobis distances (Figure 3.6) there are some similarities with that produced when male characters were not included (Figure 3.2); for example, only *Megaleledone senoi* clusters separately and sp. 17 clusters most closely with sp. 22 whilst *?Graneledone* sp. 29 clusters most closely with "*Pareledone*" *polymorpha* and "*Pareledone*" sp. 18.

Stepwise discriminant analysis excluded only three of the twenty-one indices from the data set. These were the arm length indices L1 and L3 and the opposite arm index (OAI).

Analysis of three counts taken from male specimens only of fifteen species of octopus

The non-parametric discriminant function reclassified 63% of the specimens correctly on the basis of three counts (Table 3.11), compared with 68% using eighteen indices and 82% using the same eighteen indices together with three indices derived from male characters. The phenogram of Mahalanobis distances (Figure 3.7) differed considerably from the two previous phenograms. The three smooth skinned species of *Pareledone* clustered with *Megaleledone senoi* and separately from the other *Pareledone* species. All other species formed one large cluster with no apparent distinction between higher level taxa (Figure 3.7).

Analysis of twenty-four counts and indices taken from male specimens only of fifteen species of octopus

The non-parametric discriminant function reclassified 85% of the specimens correctly (Table 3.12). As expected (since it was based on characters previously analysed separately), the phenogram of Mahalanobis distances (Figure 3.8) was a

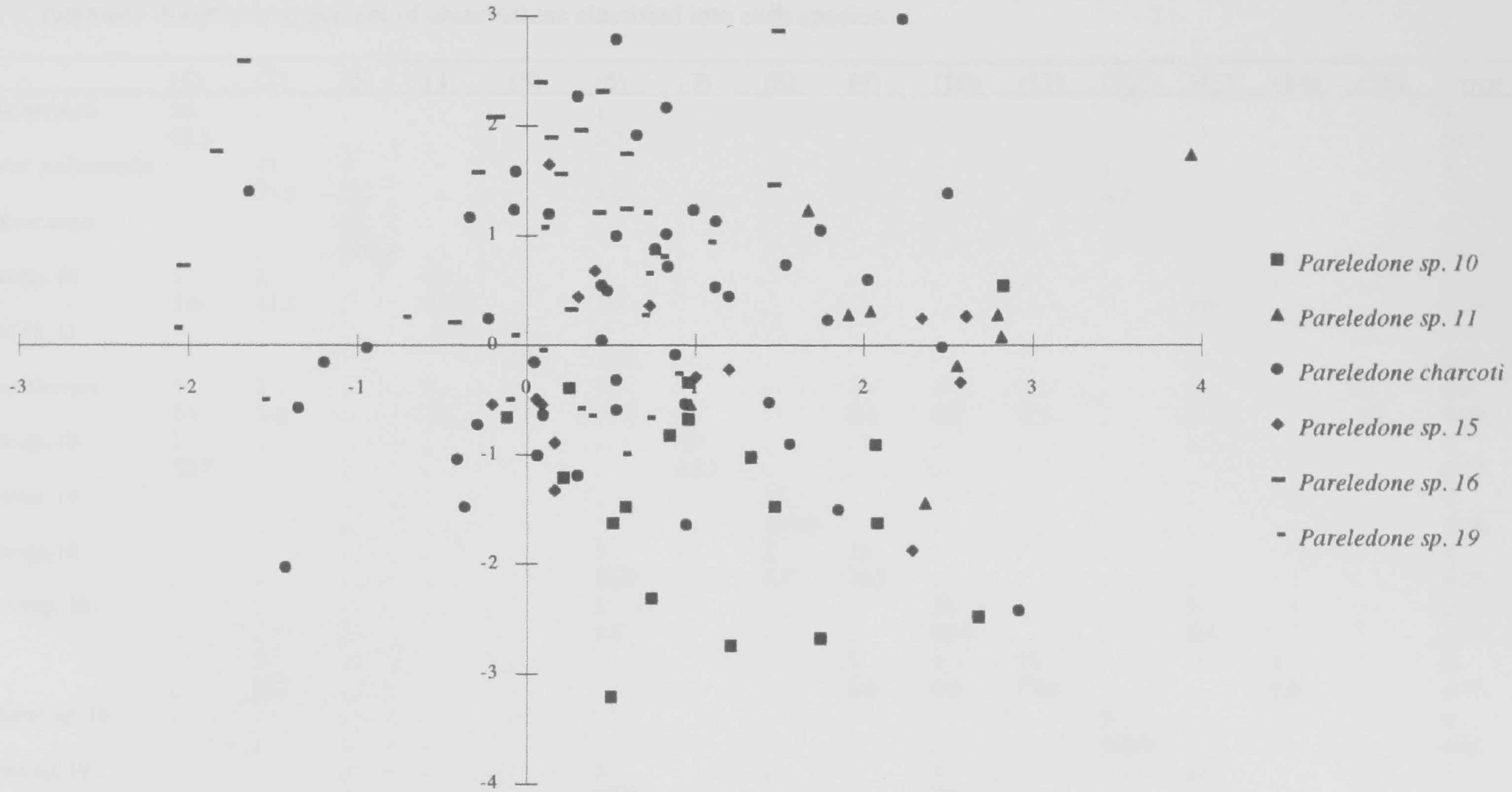


Figure 3.5: Analysis of twenty-one indices in male specimens only of fifteen species of octopus. Plot of canonical variable 1 against canonical variable 2. For clarity, only *Pareledone* species with papillose skin have been included.

Table 3.10: Analysis of twenty-one indices in male specimens only of fifteen species of octopus. Resubstitution summary using linear discriminant function. Number and percent of observations classified into each species.

Species	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	Error
(1) *Pareledone turqueti*	14	.	.	.	.	1	.	.	.	.	.	.	.	.	.	1
	93.3	.	.	.	.	6.7	.	.	.	.	.	.	.	.	.	0.07
(2) "*Pareledone*" *polymorpha*	.	17	1	.	.	1	.	.	.	2	.	1	.	.	.	5
	.	77.3	4.6	.	.	4.6	.	.	.	9.1	.	4.6	.	.	.	0.23
(3) *Megaleledone senoi*	.	.	19	.	.	.	.	.	.	.	.	.	.	.	.	0
	.	.	100.0	.	.	.	.	.	.	.	.	.	.	.	.	0.00
(4) *Pareledone* sp. 10	1	2	.	13	.	1	.	.	.	.	.	.	1	.	.	5
	5.6	11.1	.	72.2	.	5.6	.	.	.	.	.	.	5.6	.	.	0.28
(5) *Pareledone* sp. 11	.	.	.	.	6	4	.	.	.	.	.	.	.	.	.	4
	.	.	.	.	60.0	40.0	.	.	.	.	.	.	.	.	.	0.40
(6) *Pareledone charcoti*	1	1	.	1	1	34	.	.	1	2	3	.	3	.	1	14
	2.1	2.1	.	2.1	2.1	70.8	.	.	2.1	4.2	6.3	.	6.3	.	2.1	0.29
(7) *Pareledone* sp. 13	2	.	.	.	.	.	10	.	.	.	.	.	.	.	.	2
	16.7	.	.	.	.	.	83.3	.	.	.	.	.	.	.	.	0.17
(8) *Pareledone* sp. 14	.	.	.	.	.	.	.	11	.	.	.	.	.	.	.	0
	.	.	.	.	.	.	.	100.0	.	.	.	.	.	.	.	0.00
(9) *Pareledone* sp. 15	.	.	.	.	.	3	.	1	11	.	.	.	.	.	.	4
	.	.	.	.	.	20.0	.	6.7	73.3	.	.	.	.	.	.	0.27
(10) *Pareledone* sp. 16	.	.	.	.	.	1	.	.	.	16	.	.	1	.	.	2
	.	.	.	.	.	5.6	.	.	.	88.9	.	.	5.6	.	.	0.11
(11) sp. 17	.	1	.	.	.	.	.	.	1	1	14	.	.	1	.	4
	.	5.6	.	.	.	.	.	.	5.6	5.6	77.8	.	.	5.6	.	0.22
(12) "*Pareledone*" sp. 18	.	.	.	.	.	.	.	.	.	.	.	9	.	.	.	0
	.	.	.	.	.	.	.	.	.	.	.	100.0	.	.	.	0.00
(13) *Pareledone* sp. 19	.	.	.	.	.	3	.	.	.	1	.	.	13	.	.	4
	.	.	.	.	.	17.7	.	.	.	5.9	.	.	76.5	.	.	0.24
(14) sp. 22	.	.	.	.	.	.	.	.	.	.	2	.	.	3	.	2
	.	.	.	.	.	.	.	.	.	.	40.0	.	.	60.0	.	0.40
(15) ?*Graneledone* sp. 29	.	.	.	.	.	2	.	.	.	.	.	.	.	.	6	2
	.	.	.	.	.	25.0	.	.	.	.	.	.	.	.	75.0	0.25
													Overall Error Rate			0.18

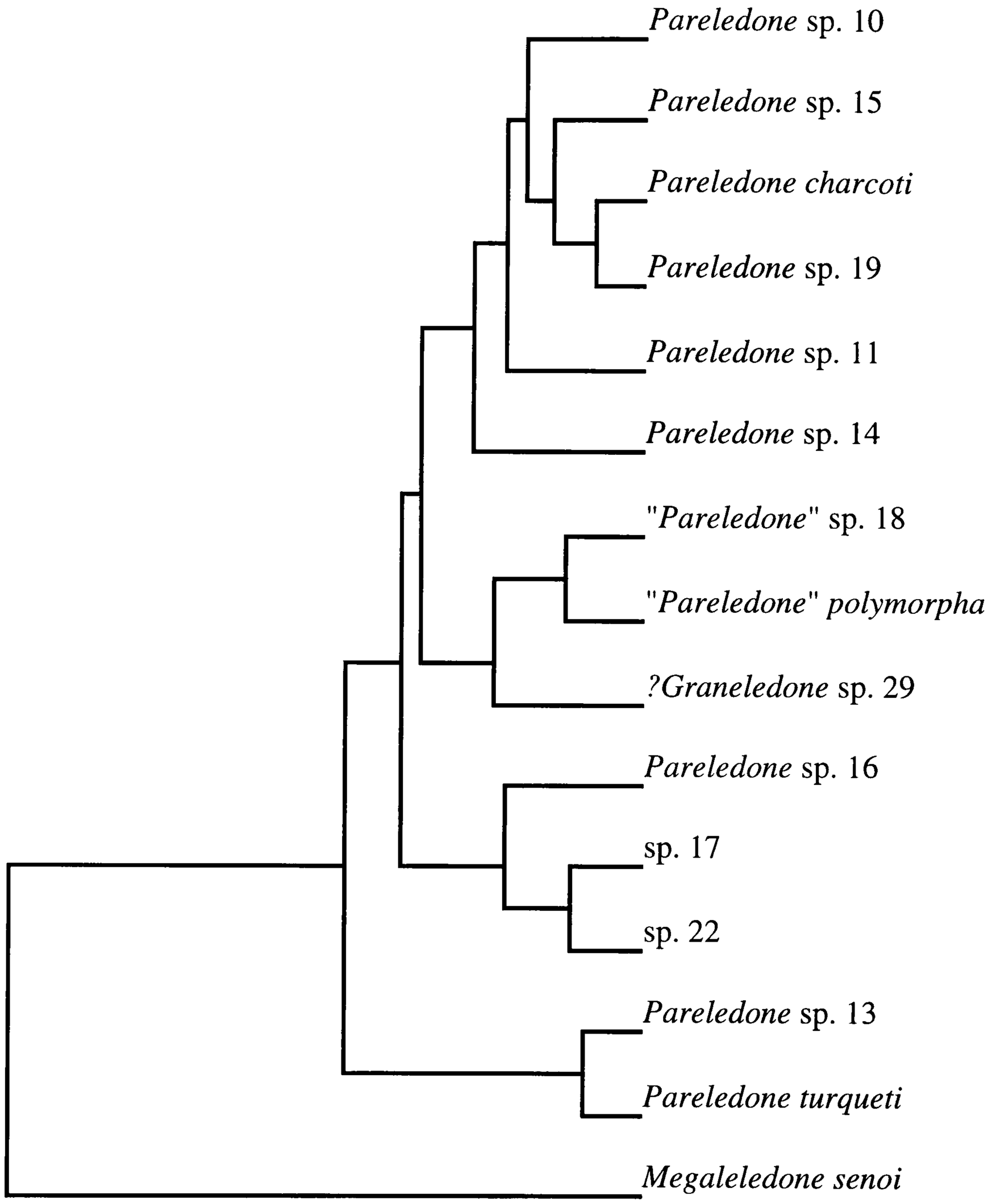


Figure 3.6: Analysis of twenty-one indices in male specimens only of fifteen species of octopus. Pairwise squared distances between species clustered using UPGMA.

Table 3.11: Analysis of three counts taken from male specimens only of fifteen species of octopus. Resubstitution summary using linear discriminant function. Number and percent of observations classified into each species.

Species	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	Other	Error
(1) *Pareledone turqueti*	29	.	1	.	.	.	4	.	.	.	.	.	2	.	.	2	9
	76.3	.	2.6	.	.	.	10.5	.	.	.	.	.	5.3	.	.	5.3	0.24
(2) "*Pareledone*" *polymorpha*	.	11	.	2	.	1	.	.	.	1	.	.	.	.	.	8	12
	.	47.8	.	8.7	.	4.4	.	.	.	4.4	.	.	.	.	.	34.8	0.52
(3) *Megaleledone senoi*	1	.	18	.	.	.	.	1	.	.	.	.	.	.	.	1	3
	4.8	.	85.7	.	.	.	.	4.8	.	.	.	.	.	.	.	4.8	0.14
(4) *Pareledone* sp. 10	.	.	.	13	.	2	.	.	1	.	.	.	.	.	.	4	7
	.	.	.	65.0	.	10.0	.	.	5.0	.	.	.	.	.	.	20.0	0.35
(5) *Pareledone* sp. 11	.	.	.	1	5	.	.	.	3	.	.	.	.	.	.	4	8
	.	.	.	7.7	38.5	.	.	.	23.1	.	.	.	.	.	.	30.8	0.62
(6) *Pareledone charcoti*	2	.	.	.	1	39	.	.	.	1	2	1	.	1	.	6	14
	3.8	.	.	.	1.9	73.6	.	.	.	1.9	3.8	1.9	.	1.9	.	11.3	0.26
(7) *Pareledone* sp. 13	.	.	.	.	.	1	22	2	.	.	.	.	.	.	.	7	10
	.	.	.	.	.	3.1	68.8	6.3	.	.	.	.	.	.	.	21.9	0.31
(8) *Pareledone* sp. 14	1	.	.	.	.	.	2	7	.	.	.	.	.	.	.	2	5
	8.3	.	.	.	.	.	16.7	58.3	.	.	.	.	.	.	.	16.7	0.42
(9) *Pareledone* sp. 15	.	1	.	.	2	.	.	.	9	.	.	.	2	.	.	4	9
	.	5.6	.	.	11.1	.	.	.	50.0	.	.	.	11.1	.	.	22.2	0.50
(10) *Pareledone* sp. 16	.	.	.	.	.	1	.	.	.	12	1	2	.	.	.	4	8
	.	.	.	.	.	5.0	.	.	.	60.0	5.0	10.0	.	.	.	20.0	0.40
(11) sp. 17	.	.	.	.	.	1	.	.	.	2	8	2	.	1	.	6	12
	.	.	.	.	.	5.0	.	.	.	10.0	40.0	10.0	.	5.0	.	30.0	0.60
(12) "*Pareledone*" sp. 18	.	.	.	.	.	1	.	.	.	1	.	7	.	.	.	4	6
	.	.	.	.	.	7.7	.	.	.	7.7	.	53.8	.	.	.	30.8	0.46
(13) *Pareledone* sp. 19	.	2	.	.	1	.	.	.	1	.	.	1	11	.	.	4	9
	.	10.0	.	.	5.0	.	.	.	5.0	.	.	5.0	55.0	.	.	20.0	0.45
(14) sp. 22	.	.	.	.	.	.	.	.	.	2	.	.	.	3	.	3	5
	.	.	.	.	.	.	.	.	.	25.0	.	.	.	37.5	.	37.5	0.63
(15) ?*Graneledone* sp. 29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	1	1
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	88.9	11.1	0.11
															Overall Error Rate		0.37

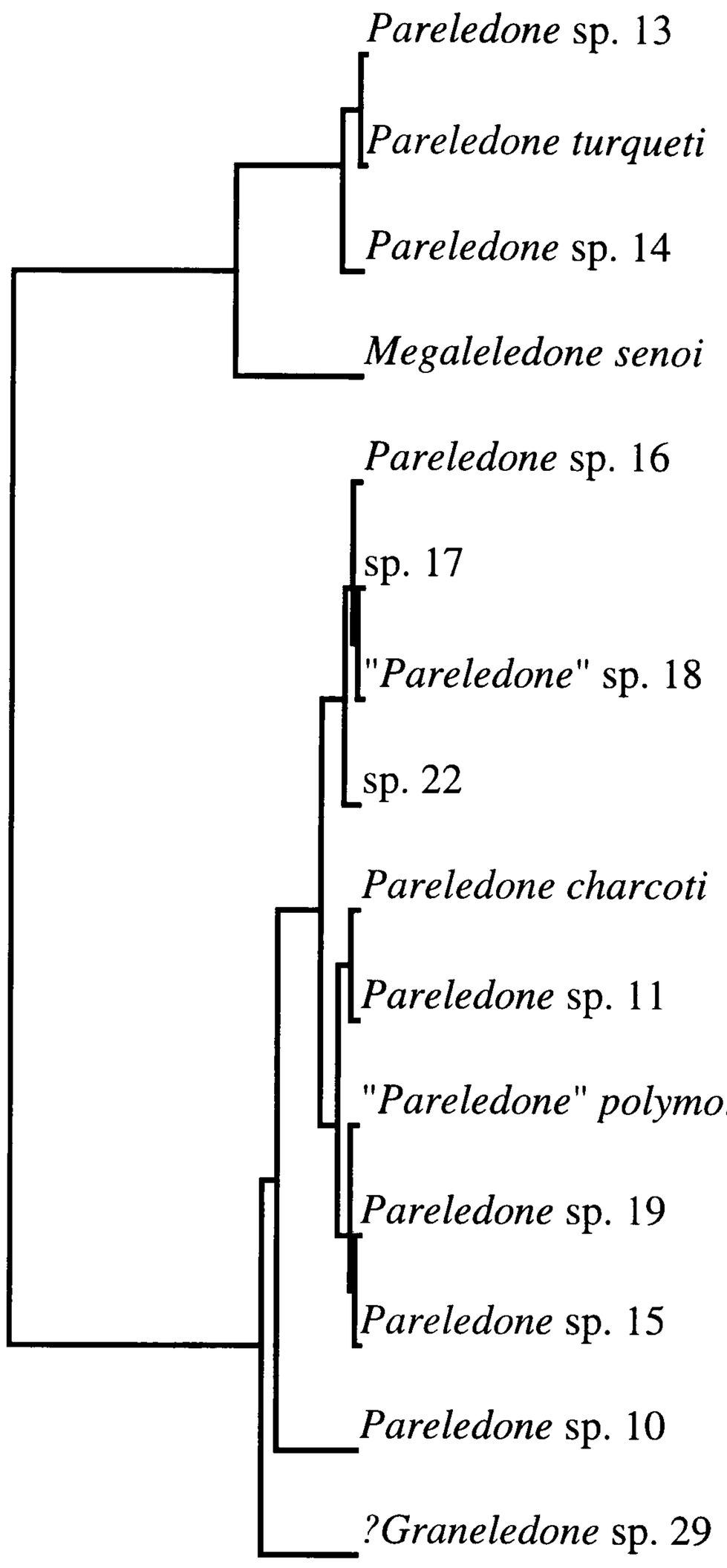


Figure 3.7: Analysis of three counts taken from male specimens only of fifteen species of octopus. Pairwise squared distances between species clustered using UPGMA.

Table 3.12: Analysis of twenty-four counts and indices taken from male specimens only of fifteen species of octopus. Resubstitution summary using linear discriminant function. Number and percent of observations classified into each species.

Species	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	Other	Error
(1) *Pareledone turqueti*	14	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	1
	93.3	.	.	.	.	.	6.7	.	.	.	.	.	.	.	.	.	0.07
(2) "*Pareledone*" *polymorpha*	.	20	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1
	.	95.2	.	.	.	.	.	.	.	.	.	.	.	.	.	4.76	0.05
(3) *Megaleledone senoi*	.	.	20	.	.	.	.	.	.	.	.	.	.	.	.	.	0
	.	.	100.0	.	.	.	.	.	.	.	.	.	.	.	.	.	0.00
(4) *Pareledone* sp. 10	.	.	.	13	.	1	.	.	.	.	.	.	.	.	.	4	5
	.	.	.	72.2	.	5.6	.	.	.	.	.	.	.	.	.	22.2	0.28
(5) *Pareledone* sp. 11	.	.	.	.	7	.	.	.	.	.	.	.	1	.	.	2	3
	.	.	.	.	70.0	.	.	.	.	.	.	.	10.0	.	.	20.0	0.30
(6) *Pareledone charcoti*	.	.	.	.	.	40	.	.	.	1	.	.	1	.	.	6	8
	.	.	.	.	.	83.3	.	.	.	2.1	.	.	2.1	.	.	12.5	0.17
(7) *Pareledone* sp. 13	.	.	.	.	.	.	11	.	.	.	.	.	.	.	.	1	1
	.	.	.	.	.	.	91.7	.	.	.	.	.	.	.	.	8.3	0.08
(8) *Pareledone* sp. 14	.	.	.	.	.	.	.	11	.	.	.	.	.	.	.	.	0
	.	.	.	.	.	.	.	100.0	.	.	.	.	.	.	.	.	0.00
(9) *Pareledone* sp. 15	.	.	.	.	.	.	.	.	11	.	.	.	1	.	.	3	4
	.	.	.	.	.	.	.	.	73.3	.	.	.	6.7	.	.	20.0	0.27
(10) *Pareledone* sp. 16	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.	2	2
	.	.	.	.	.	.	.	.	.	88.9	.	.	.	.	.	11.1	0.11
(11) sp. 17	.	.	.	.	.	.	.	.	.	1	11	.	.	2	.	4	7
	.	.	.	.	.	.	.	.	.	5.6	61.1	.	.	11.1	.	22.2	0.39
(12) "*Pareledone*" sp. 18	.	.	.	.	.	.	.	.	.	.	.	8	.	.	1	.	1
	.	.	.	.	.	.	.	.	.	.	.	88.9	.	.	11.1	.	0.11
(13) *Pareledone* sp. 19	.	.	.	.	.	.	.	.	.	.	.	.	14	.	.	3	3
	.	.	.	.	.	.	.	.	.	.	.	.	82.4	.	.	17.7	0.18
(14) sp. 22	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	1	1
	.	.	.	.	.	.	.	.	.	.	.	.	.	80.0	.	0.20	0.20
(15) ?*Graneledone* sp. 29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	.	0
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	100.0	.	0.00
															Overall Error Rate		0.15

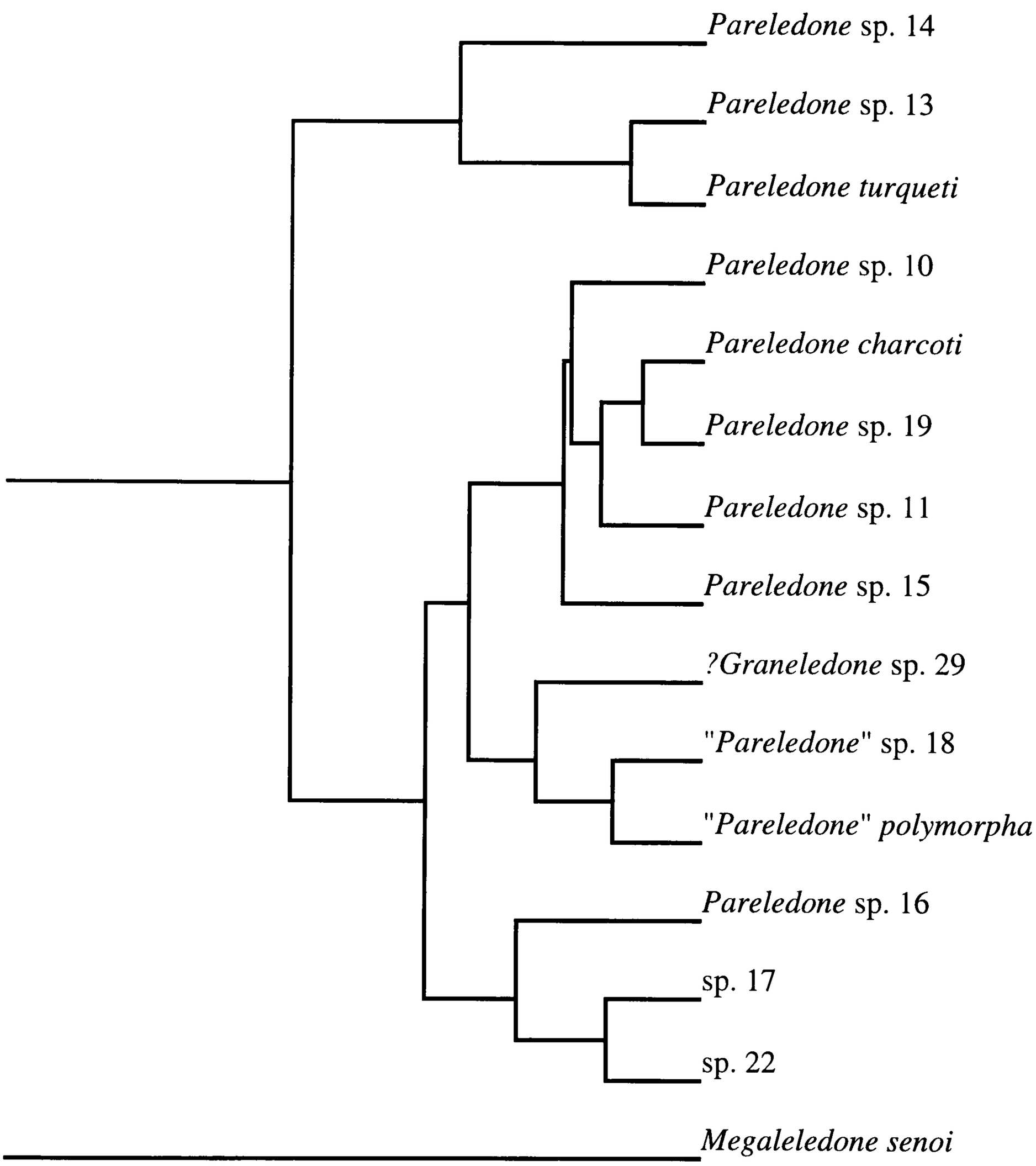


Figure 3.8: Analysis of twenty-four counts and indices taken from male specimens only of fifteen species of octopus. Pairwise squared distances between species clustered using UPGMA. *Megaleledone senoi* clusters too distantly to be portrayed at this scale.

hybrid of the previous phenograms. *Megaleledone senoi* was clearly separated from the other species. Sp. 17 and sp. 22 clustered together and closely with *Pareledone* sp. 16, whilst *?Graneledone* sp. 29 clustered most closely with "*Pareledone*" *polymorpha* and "*Pareledone*" sp. 18. The papillose species of *Pareledone* adjoin these clusters closely, whereas the smooth skinned *Pareledone* cluster together and distantly. The four phenograms have all been illustrated on the same scale and this phenogram, using all twenty-four indices and counts, shows the greatest separation between species.

Analysis of allozyme electrophoretic data from fifteen species of octopus

In the dendrogram of Nei's (1978) genetic identity (Figure 3.9), *Megaleledone senoi* clusters distantly from all other species. There is one large cluster containing all the *Pareledone.* Within this are two smaller clusters; one contains the smooth skinned *Pareledone*, the other contains all the papillose *Pareledone.* Sp. 22 is also included in the latter. "*Pareledone*" *polymorpha* and "*Pareledone*" sp. 18 cluster together, but some distance from the true *Pareledone.* In contrast to the phenograms based on Mahalanobis distance, sp. 17 is clearly distant from sp. 22.

Analysis of seven indices calculated from beak measurements of nine species of octopus

Canonical discriminant analysis, based on seven indices derived from beak measurements, produced seven canonical variates; the first three canonical variates accounted for 87% of the total variance (Table 3.13). All the indices had high character loadings on at least one of the first three canonical variates. A plot of the first two canonical variates (Figure 3.10) shows that *Octopus tehuelchus*, *Benthoctopus eureka* and "*Pareledone*" *polymorpha* are distinguishable, whereas *Megaleledone senoi* and all the *Pareledone* scatter together. The discriminant function reclassified 59% of the specimens correctly (Table 3.15). In the phenogram of Mahalanobis distances (Figure 3.11) all species of *Pareledone* cluster together;

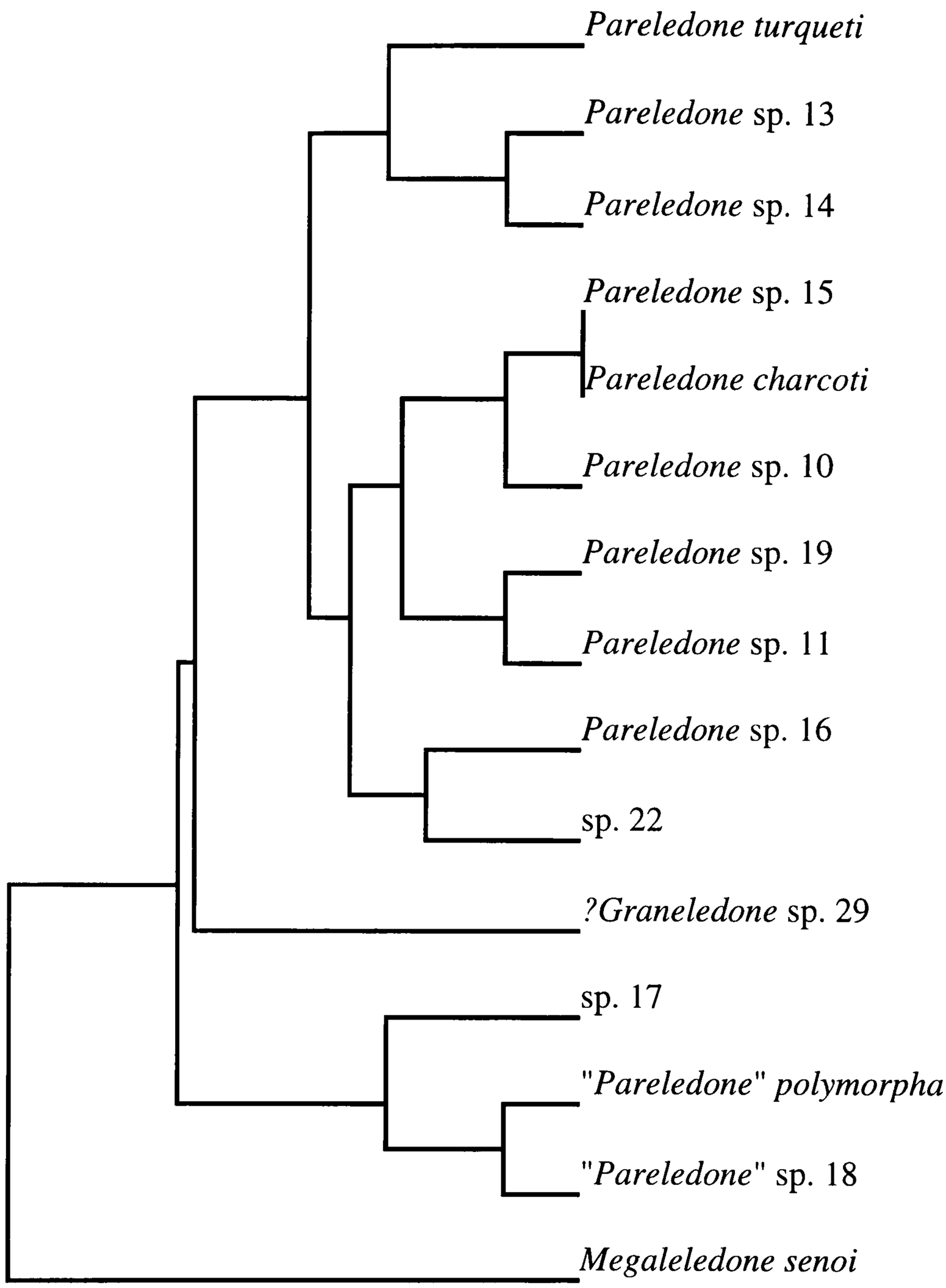


Figure 3.9: Analysis of allozyme electrophoretic data from fifteen species of octopus. Pairwise squared distances (*I*; Nei, 1978) between species clustered using UPGMA.

Table 3.13: Analysis of seven indices calculated from beak measurements of nine species of octopus. Eigenvalues of the correlation matrix calculated by canonical correlation.

Canonical Variate	Eigenvalue	Proportion of Variance	Cumulative Proportion
1	2.3771	0.5759	0.5759
2	0.8448	0.2047	0.7806
3	0.3716	0.0900	0.8707
4	0.2192	0.0531	0.9238
5	0.1896	0.0459	0.9697
6	0.0963	0.0233	0.9930
7	0.0288	0.0070	1.0000

Table 3.14: Analysis of seven indices calculated from beak measurements of nine species of octopus. Total-sample standardized canonical coefficients.

Index	Coefficient 1	Coefficient 2	Coefficient 3
WLI	0.399	-0.231	1.539
HI	0.722	0.378	-0.664
RPI	0.691	0.165	-0.279
HLI	0.106	1.366	0.071
CLI	0.351	0.317	-1.051
REI	0.249	-0.288	1.270
BLI	0.410	-0.172	0.018

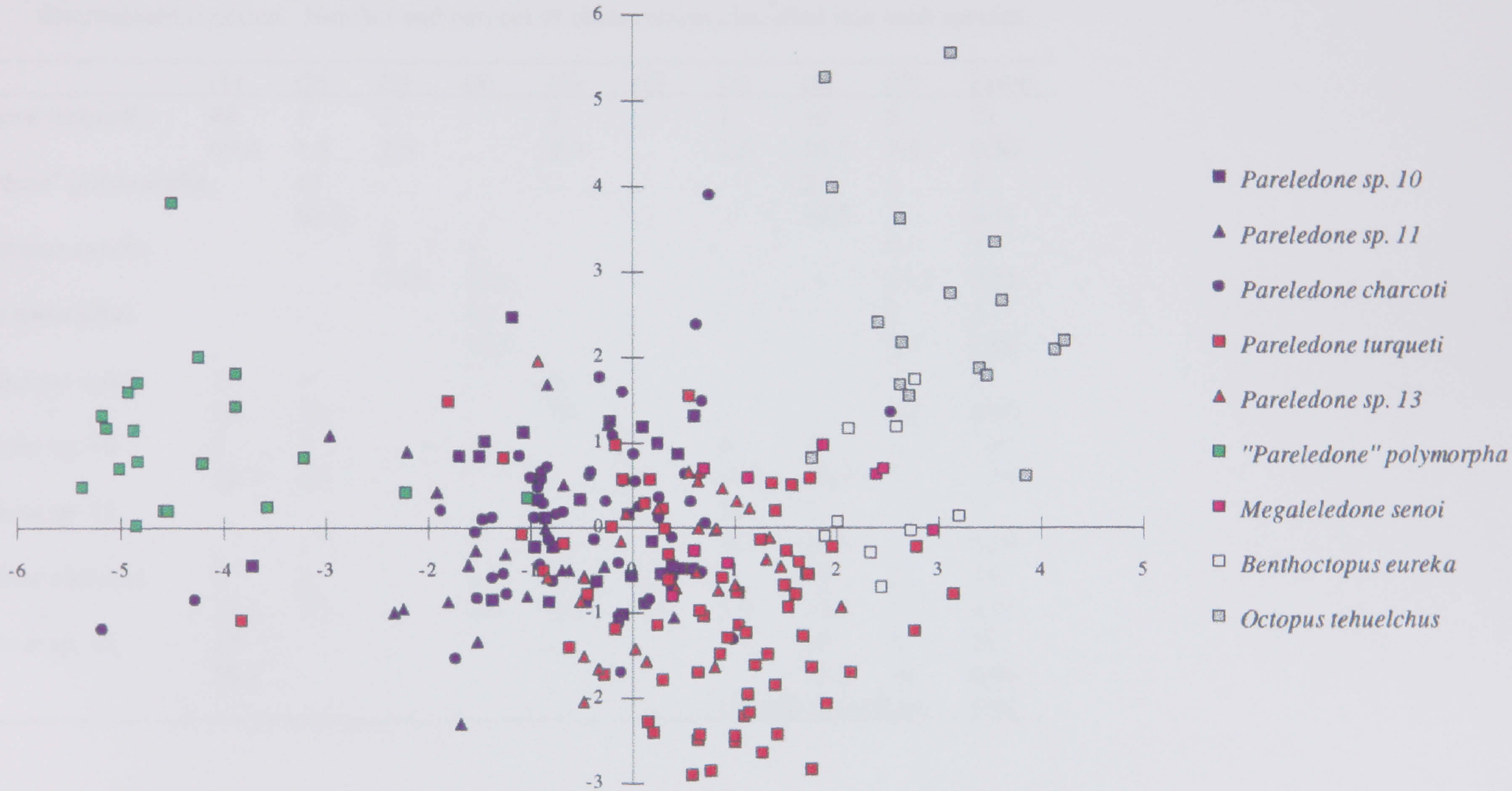


Figure 3.10: **Analysis of seven indices calculated from beak measurements of nine species of octopus. Plot of canonical variable 1 against canonical variable 2.**

Table 3.15: Analysis of seven indices calculated from beak measurements of nine species of octopus. Resubstitution summary using linear discriminant function. Number and percent of observations classified into each species.

Species	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	Error
(1) *Pareledone turqueti*	48	1	2	.	2	.	1	10	5	21
	69.6	1.5	2.9	.	2.9	.	1.5	14.5	7.3	0.30
(2) "*Pareledone*" *polymorpha*	.	17	.	.	.	.	.	2	.	2
	.	89.5	.	.	.	.	.	10.5	.	0.11
(3) *Benthoctopus eureka*	.	.	7	1	.	.	.	.	1	2
	.	.	77.8	11.1	.	.	.	.	11.1	0.22
(4) *Octopus tehuelchus*	.	.	.	16	.	.	.	.	1	2
	.	.	.	94.1	.	.	.	.	5.9	0.06
(5) *Megaleledone senoi*	2	1	.	.	6	.	.	.	1	3
	20	10	.	.	60	.	.	.	10	0.40
(6) *Pareledone* sp. 10	6	1	.	.	.	.	4	17	1	29
	20.7	3.5	.	.	.	.	13.8	58.6	3.5	1.00
(7) *Pareledone* sp. 11	.	.	.	.	.	.	16	5	.	5
	.	.	.	.	.	.	76.2	23.8	.	0.24
(8) *Pareledone charcoti*	6	2	.	1	1	1	1	40	2	14
	11.1	3.7	.	1.9	1.9	1.9	1.9	74.1	3.7	0.26
(9) *Pareledone* sp. 13	17	.	.	.	.	.	.	10	3	26
	56.7	.	.	.	.	.	.	33.3	10	0.90
							Overall Error Rate			0.41

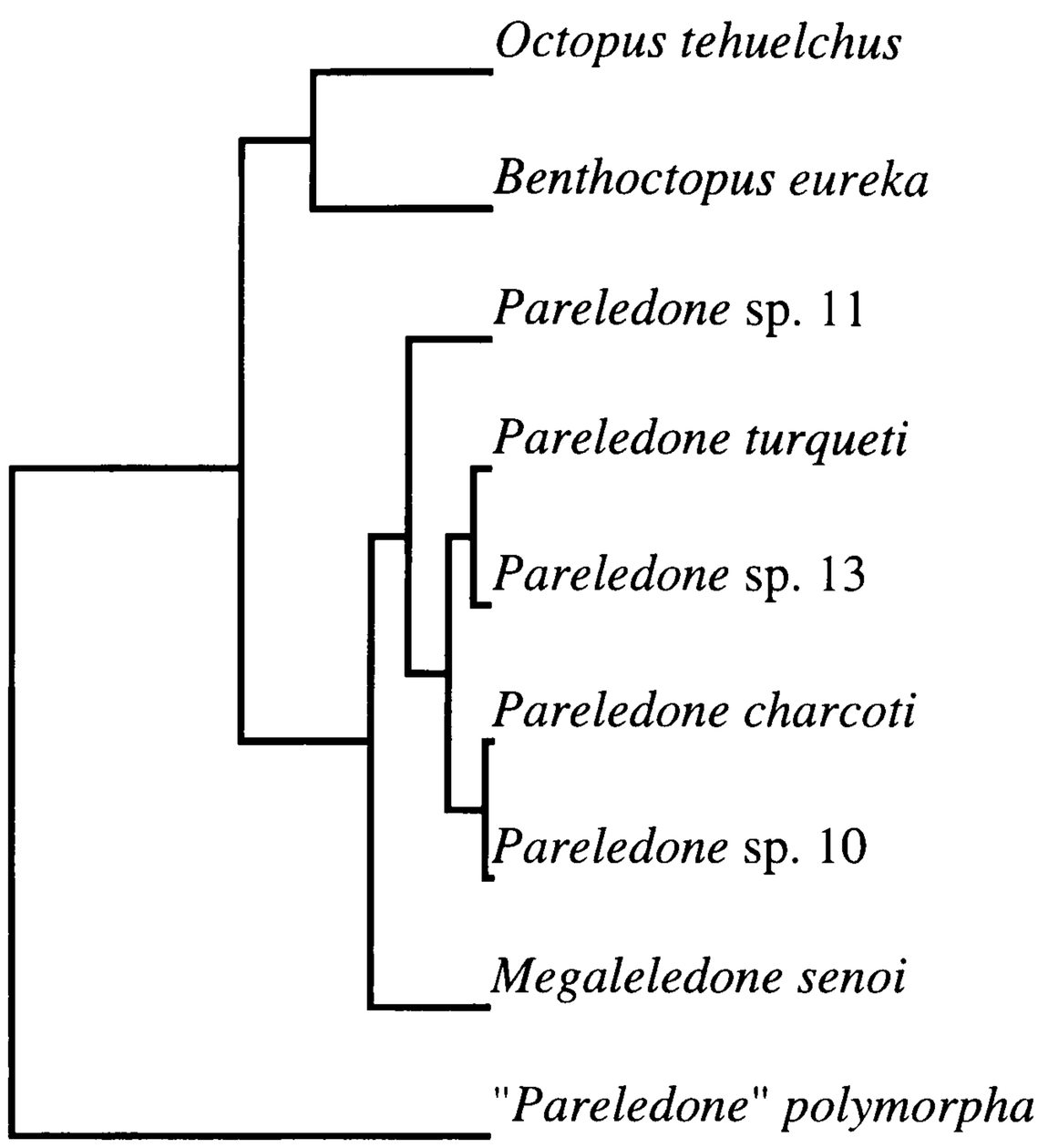


Figure 3.11: Analysis of seven indices calculated from beak measurements of nine species of octopus. Pairwise squared distances between species clustered using UPGMA.

Megaleledone senoi is closely associated with these in contrast to the other three species. The five *Pareledone* species also cluster closely in the dendrogram constructed from Nei's (1978) genetic identity (Figure 3.12). The major difference is that in the latter *Megaleledone senoi* is not closely associated with the *Pareledone*.

Stepwise discriminant analysis did not exclude any of the indices from the data set.

Discussion

Analysis of indices taken from fifteen species of octopus

When only females (and therefore no characters specific to males) were included in the analysis, web depth index (WDI), mantle arm index (MAI) and funnel length index (FuLI) stood out as useful characters for discriminating between species. It is of little surprise that WDI and MAI vary between species as these are both characters that are likely to reflect the lifestyle of the species; it is likely that web depth and relative arm lengths have evolved to suit the motility and predatory actions of each species. On the interior surface of the funnel musculature lies the funnel organ. The shape of this organ is thought to be particularly important in cephalopod systematics (Roper and Voss, 1983). It is possible that the size of the funnel itself is a reflection of the size and shape of the funnel organ and this would explain the importance of FuLI as a discriminating character.

When characters specific to males were included in the analyses the arm length indices L3 and R3, and the opposite arm index (OAI) appeared more important than MAI and FuLI. L3 is actually a very similar index to MAI (L3, length of third left arm as a percentage of the dorsal mantle length; MAI, dorsal mantle length as a percentage of the longest arm) and a matrix of correlations (not shown) indicated highly significant correlation between these two indices. The third right arm is the hectocotylised arm in the male. Since reproductive success is directly dependent on the function of this modified arm it is unsurprising that the length of

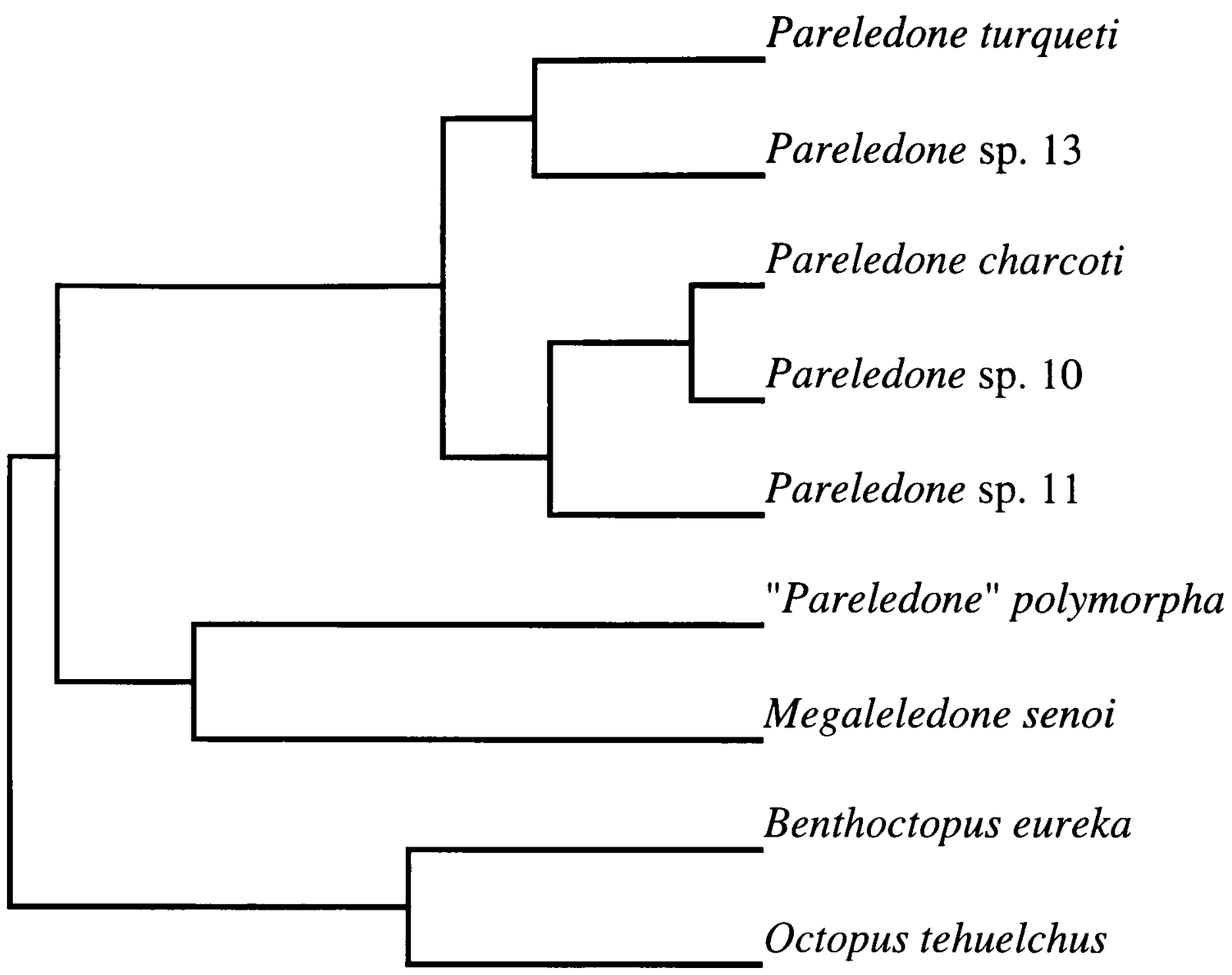


Figure 3.12: Analysis of allozyme electrophoretic data from nine species of octopus. Pairwise squared distances (*I*; Nei, 1978) between species clustered using UPGMA.

this arm is species specific and that two indices which involve this measurement (R3 and OAI; see Table 3.1) are important as discriminating characters.

The addition of three indices specific to males increased the power of the discriminant function by fourteen percentage points, even though fewer specimens were used to determine the function. Because, as mentioned above, reproductive success is partially dependent upon many characters confined to males, there is probably less variation within species in these characters and possibly more variability between species in similar environments (because of increased genetic and decreased environmental effects). Clearly male characteristics have an important role to play in species discrimination and should always be included in species descriptions.

Since the first two canonical variables accounted for less than 60% of the variance, the discriminating power of the function is not easily illustrated (Figures 3.3 - 3.5). Although in theory a three dimensional scatter plot (accounting for 71% of the variance) would show greater discrimination, in practice there were too many data points and the resulting plot was less clear. However, even from the two-dimensional plot there is some evidence of separation at the specific level and the evidence for separation at higher levels is clear. Discrimination at the specific level is supported by the fact that 82% of specimens were reclassified correctly by the function. It must be borne in mind, however, that ideally one would have two data sets; one of these would be used to derive the discriminant function whilst the other would be used to test it. Clearly the percentage of specimens reclassified correctly will be higher if the same data set is used for both purposes.

Stepwise discriminant function analysis (SDFA) excluded the arm length indices L1 and L3, and the opposite arm index (OAI) from the function, although L3 and OAI were both indices with high character loadings. A correlation matrix produced during the analysis (not shown) indicated that all arm length indices were highly correlated with one another and that OAI was highly correlated with L3 and R3, the indices from which it is derived. Therefore, SDFA is merely excluding data

that have already effectively been duplicated. This suggests that all of the measurements taken were important in increasing the power of the discriminant function, but that care should be taken when converting these measurement to indices.

Analysis of counts taken from fifteen species of octopus

As the counts were analysed non-parametrically the test results are not directly comparable with those from the analysis of indices; parametric tests are normally considered to be more powerful. However, counts appear to be important and effective characters for discriminating between three species, as a discriminant function based on three counts reclassified only 5% fewer specimens correctly, than did a function based on eighteen indices. In the phenogram the smooth skinned *Pareledone* and *Megaleledone senoi* cluster distantly from the other species. This is probably due to differences in the numbers of gill lamellae; smooth skinned *Pareledone* and *Megaleledone* have 9-13 gill lamellae whereas the other species have 6-9.

When the indices are combined with the counts the greatest number of specimens are reclassified correctly by the discriminant function. This emphasizes the need to take as many measurements as possible. Apart from the indices used here, Roper and Voss (1983) also suggest the use of calamus length index, spermatophore width index, spermatophore length index, penis length index, penis diverticulum length index and egg length index. Calamus length index was initially calculated in this study but it was discarded as the calamus is so small in the genus *Pareledone* that it was impossible to measure it accurately on fresh specimens. The other five indices are highly dependent on maturity stage and too few fully mature animals were collected to be able to test these indices as part of this study. However, in the light of the results of this study, there is little doubt that these indices would be useful and details on spermatophore and egg sizes in mature individuals are given in species descriptions (Chapter 4).

Interpretation of phenograms produced from indices and counts

No cluster patterns were consistent in all four of the phenograms produced. This illustrates the importance of character choice when producing phylogenies. Ideally, to produce a phenogram that reflects evolutionary relationships, characters must be chosen that are homologous rather than analogous, and in cephalopods much of this information on characters is lacking.

The use of phenetics in elucidating phylogenies has been widely criticised since the advent of cladistic theory (Hennig, 1966) and Voight (1993b) suggests that cladistics is the only way forward in octopodid taxonomy. Phenetic methods are still widely used though, and their usefulness has been demonstrated in recent phylogenetic research (Hageman, 1991; Jackson and Cheetham 1994; Lafay *et al.*, 1995; Zelditch *et al.*,1995; Rohlf *et al.*, 1996).

There were some similarities between the phenograms. For instance, sp. 17 and sp. 22 clustered together in three of the phenograms and closely in the fourth and hence appear to be morphologically similar, although their overall similarity is refuted by the electrophoretic data. *?Graneledone* sp. 29 groups closely with "*Pareledone*" *polymorpha* and "*Pareledone*" sp. 18 on the phenograms, although detailed inspection of the beak, hectocotylus, salivary glands and internal chromatophore patterns reveal that these bear greater resemblance to *Pareledone* species than to "*P.*" *polymorpha* and "*P.*" sp. 18. However, the latter two species themselves often clustered within the *Pareledone* on the basis of general shape even though they are known to differ widely on specific characters (see Chapter 1). This illustrates again, how general characters of shape may be misleading, and sheds no light on the correct classification of *?Graneledone* sp. 29, sp. 17 and sp. 22.

The only consistent character of the phenograms which agrees with the dendrogam of genetic identity is the separation of *Megaleledone senoi* from the other species; this supports the views of Kubodera and Okutani (1986, 1994) and Lu and Stranks (1994) that the genus is valid. In general, however, whilst the characters utilised in the study are good for discriminating between species, and their inclusion

in taxonomic descriptions is essential, these characters are of little use in elucidating taxonomic relationships.

Analysis of morphometric indices calculated from beaks of nine species of octopus

Fewer specimens were reclassified using beak morphometrics than using other indices or counts. Character loadings stress the importance of all the indices to the discriminant function and this is supported by the inclusion of all the indices in the stepwise analysis. It is possible that had further beak measurements been taken, the discrimination would have improved. However, it is clear that beak morphology is unlikely to prove as useful in octopodids as it is in squid.

Although these indices do not discriminate well at the specific level there is evidence from the re-substitution summary (Table 3.15) and from the scatter plot of the first two canonical variates (Figure 3.10) that better separation is achieved at the generic level. The clear separation of "*Pareledone*" *polymorpha* from the other *Pareledone* species lends further support to the suggestion that this species should be removed from the genus.

There is reasonable agreement between the phenogram and the dendrogram of genetic identity suggesting that beak structure may be useful in reconstructing octopus phylogenies. However, analysis of more species from more genera is required to confirm this. The usefulness of beak morphology as a taxonomic character at the generic level in Antarctic octopodids is explored more fully in Ogden *et al.* (in press).

Chapter 4

Taxonomy of Southern Ocean Octopodidae with descriptions of seven new species of *Pareledone* from Western Antarctica.

Introduction

Recent research that was undertaken to provide systematic information on the Octopodidae of Eastern Antarctica has led to the discovery of two new species of the genus *Pareledone* (Lu and Stranks, 1994). These authors published information on all the species in the area, giving comparisons of all the valid species. Several of the species were previously only known from type material, and a major review of the genus had not occurred since Robson's (1932) monograph.

In Western Antarctica there is need of a similar review. Although there has been recent work on the *Pareledone* in this area it has not all been of a taxonomic nature (e.g. Kuehl, 1988; Daly, 1996). The taxonomic work in the region has centred mainly upon other genera e.g., *Graneledone* (Voss, 1976; Kubodera and Okutani, 1994), *Megaleledone* (Kubodera and Okutani, 1994), *Thaumeledone* (Stranks *et al.*, in prep) and *Bentheledone* (see Voss, 1988b). There is only one recent study of the *Pareledone* from the South Shetland Islands (Antarctic Peninsula) and this suggested that "*Pareledone*" *adeliana* and *Pareledone harrissoni*, previously thought to be restricted to Eastern Antarctica, are circumpolar (Kubodera and Okutani, 1994). Because of the synopsis of Lu and Stranks (1994), good descriptions of "*P.*" *adeliana* and *P. harrissoni* are now available, and it should be easier to establish whether these species really do have such an extensive range.

The primary aim of this chapter was to produce a synopsis of the *Pareledone* species of Western Antarctica, similar to that produced by Lu and Stranks (1994) for Eastern Antarctica, and to compare the fauna of the two regions.

With the focus of this thesis on *Pareledone,* it was undesirable to consider in detail those species that do not belong to the genus. However, many interesting and often rare specimens were obtained during the course of this study, and although several of the species examined are currently being described or redescribed by other authors (see below), it was felt that some description, at least, was required of all the new Antarctic specimens. The aim was not to make detailed comments on the

systematic position of any of these species, but simply to make them clearly recognisable to subsequent researchers in the field.

Methods

Four sampling programmes yielded substantial collections of benthic octopodids (Chapter 2, Figure 2.2). At South Georgia, in the Weddell Sea and at the Antarctic Peninsula live octopuses were trawled and observed aboard ship. These were subsequently killed in fresh water and examined immediately. Specimens from the Falkland Islands were not observed live and the specimens examined had previously been frozen. Over 3,500 octopuses were captured and taxonomic counts and measurements were recorded from approximately 650 specimens. Where possible, detailed measurements were taken from 20 mature males and 20 mature females, but in many cases fewer animals than this were examined. Specimens were sexed (M, male; F, female) and assigned a maturity stage from 1 to 3 (1, immature; 2, submature; 3, mature). Counts and indices were defined by Roper and Voss (1983) and are briefly described in Chapter 3 (Table 3.1). The arithmetic mean and the range is given for all indices. A standard format is used throughout: (INDEX lower range-mean-upper range). The range is also given for gill lamellae counts; the modes of inner and outer gill lamellae counts are given separately. Web formulae and arm formulae are given in the standard format suggested by Roper and Voss (1983) whereby web sector and arm lengths are expressed numerically in decreasing order. Specimen abbreviations are as follows: BMNH, British Museum of Natural History; WS, specimens examined during cruise of *PFS Polarstern* in the Weddell Sea; AP, specimens examined during cruise of *PFS Polarstern* off the Antarctic Peninsula. Many of the specimens prefixed WS and AP are currently held at British Antarctic Survey, Cambridge, UK.

Results

Notes on the identification of Southern Ocean Octopodidae

The genus *Pareledone*, together with species previously associated with it, is considered in depth below and seven new species are described. There were, though, apparently eight new species. One of these, *Pareledone* sp. 26 (Plate 4.1), was represented by only four specimens and this was not considered a large enough sample upon which to base a description. The validity of *Pareledone* sp. 26 was confirmed by electrophoresis (see Chapter 2) and it can be distinguished from other members of the genus simply by its integumental sculpture. All four specimens were very similar and that illustrated is therefore typical. The papillae are exceptionally small, both in width and height, and are closely spaced. The shallow web and the grey colouration with large white markings are distinctive.

Megaleledone senoi (Plate 4.2) is well described by Taki (1961), reviewed by Lu and Stranks (1994), and data provided in Chapters 2 and 3 confirm the validity of the genus *Megaleledone*. Mature animals are distinctive because of their large size; they may reach a total length of more than 750 mm. Immature specimens are recognisable by their deep web and, on dissection, by the large number of gill lamellae (9-14). One specimen was of particular interest. A mature female of mantle length 230 mm was captured and the ripe ovary of this specimen contained eggs of 29 mm x 11 mm which are larger than those previously described (c.f. 17 mm x 5.5 mm (Taki, 1961) and 19 mm x 8 mm (Lu and Stranks, 1994)). It is difficult to assess when a female octopodid has reached maximum maturity and it is possible that directly prior to laying, eggs may be even larger than 29 mm.

Four species were closely associated with the *Pareledone* during the analyses in Chapters 2 and 3 and it is unclear to which genera these should be ascribed. Sp. 22 (Figure 4.1) and sp. 17 (Plate 4.3) were superficially similar (Chapter 3) with deep webs, gelatinous skin, and simple hectocotyli, although electrophoresis indicated they were not particularly closely related (Chapter 2). *?Graneledone* sp. 25 was similar in general shape to the *Pareledone* but lacked an ink sac. It was tentatively assigned to

Plate 4.1: *Pareledone* sp. 26: 34 mm ML.

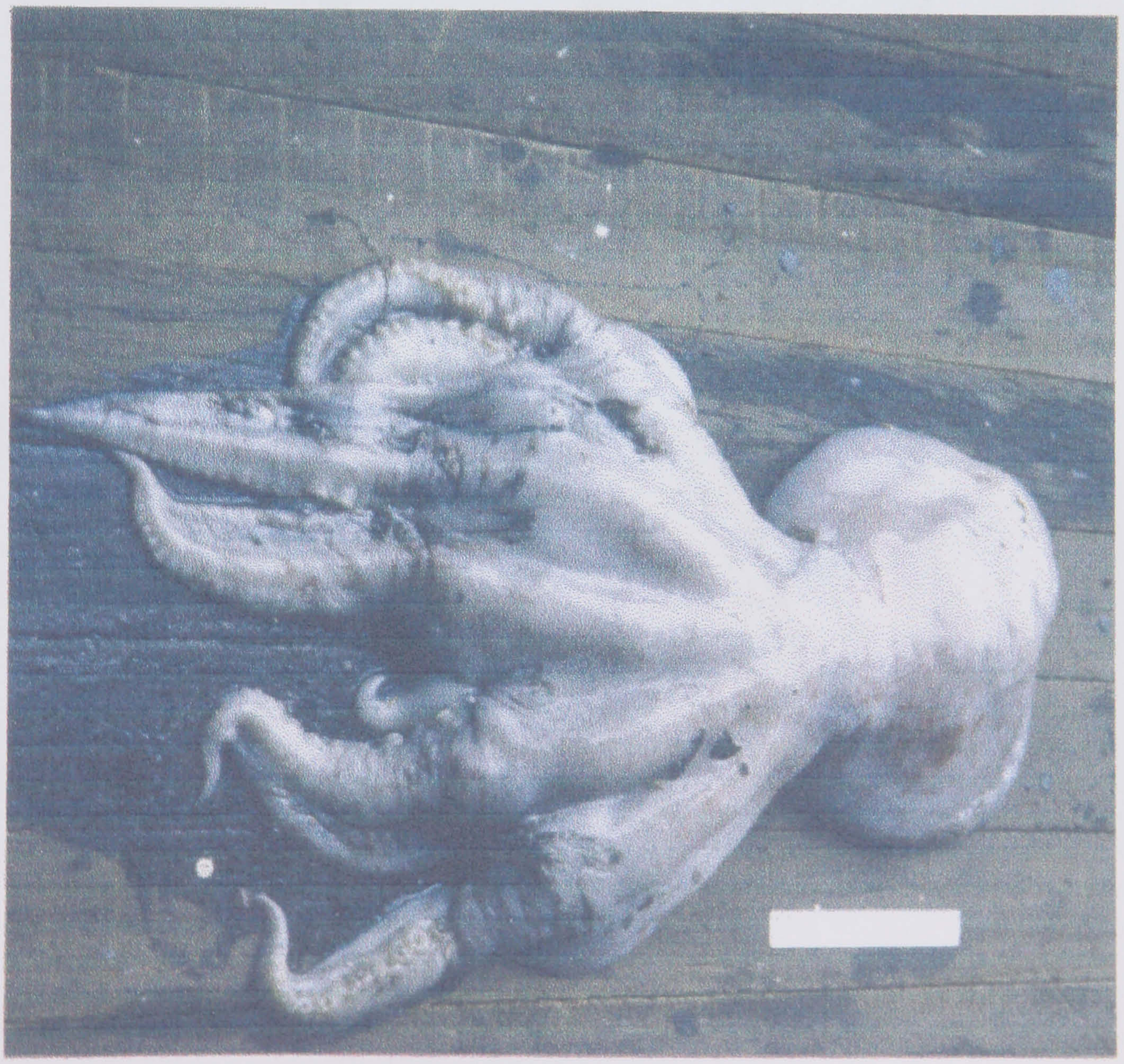


Plate 4.2: *Megaleledone senoi*: 230 mm ML.

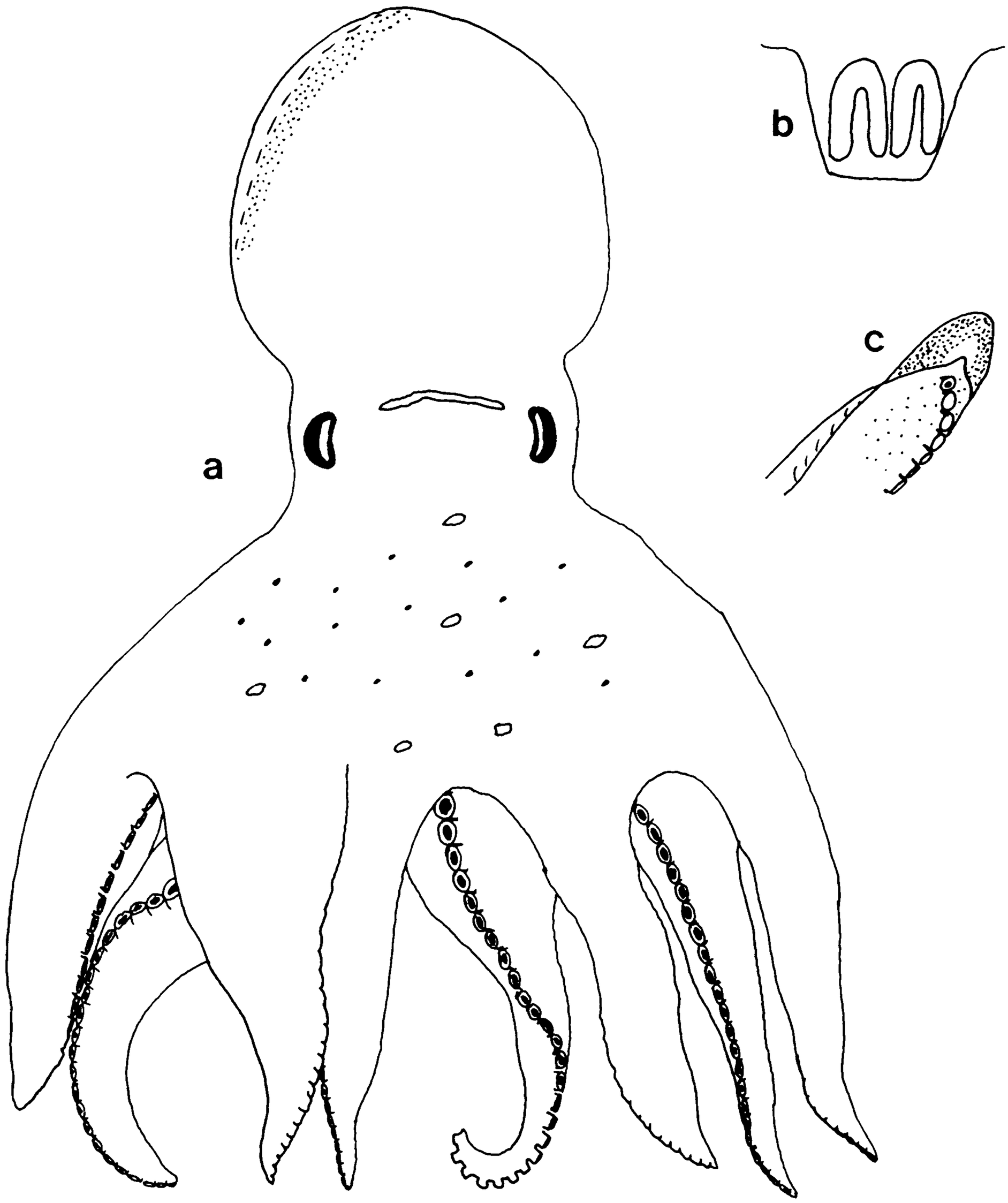


Figure 4.1a-c: Sp. 22: a, dorsal view of whole animal b, funnel organ c, hectocotylus. All illustrations actual size.

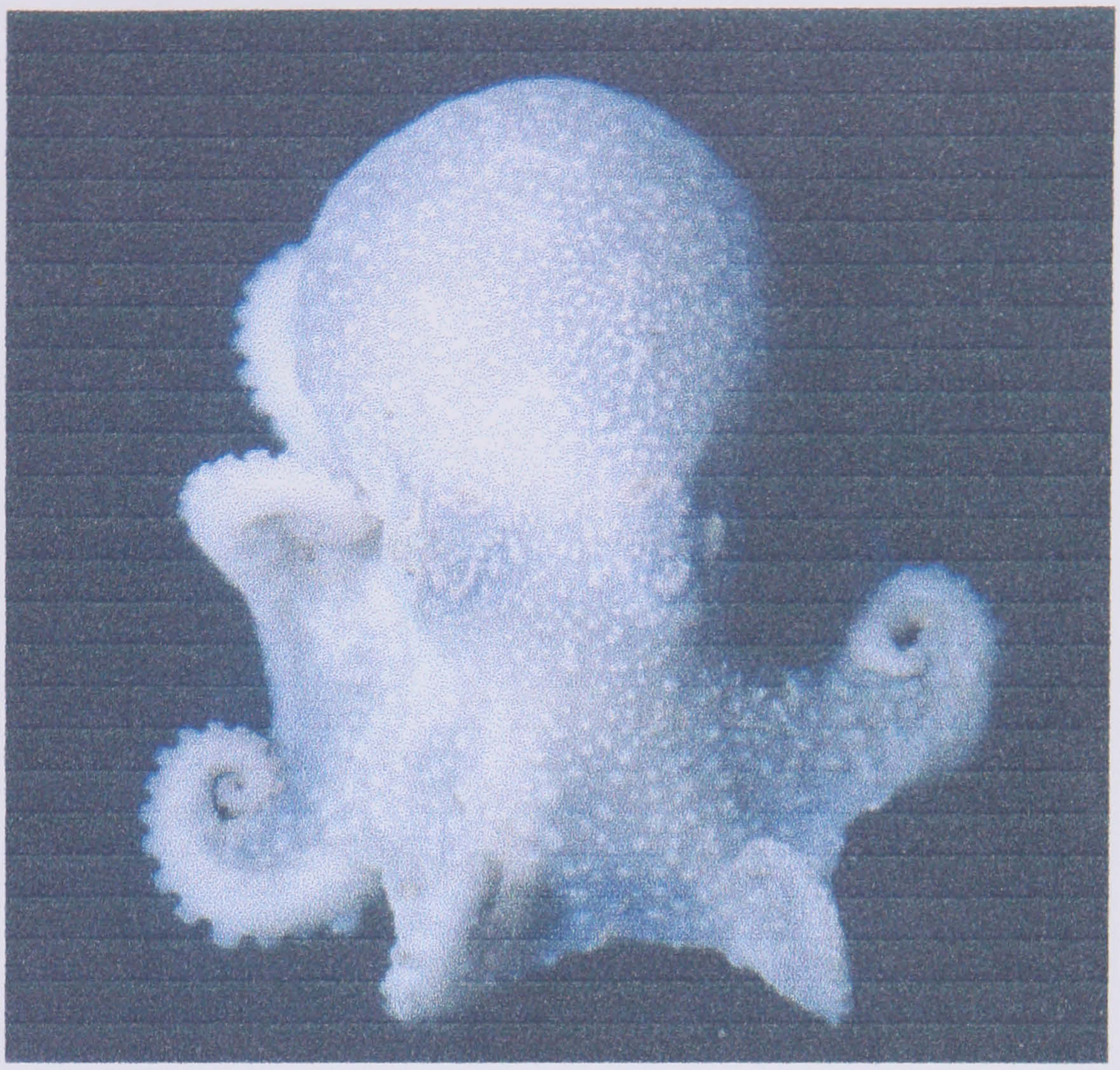


Plate 4.3: Sp. 17: 57 mm ML.

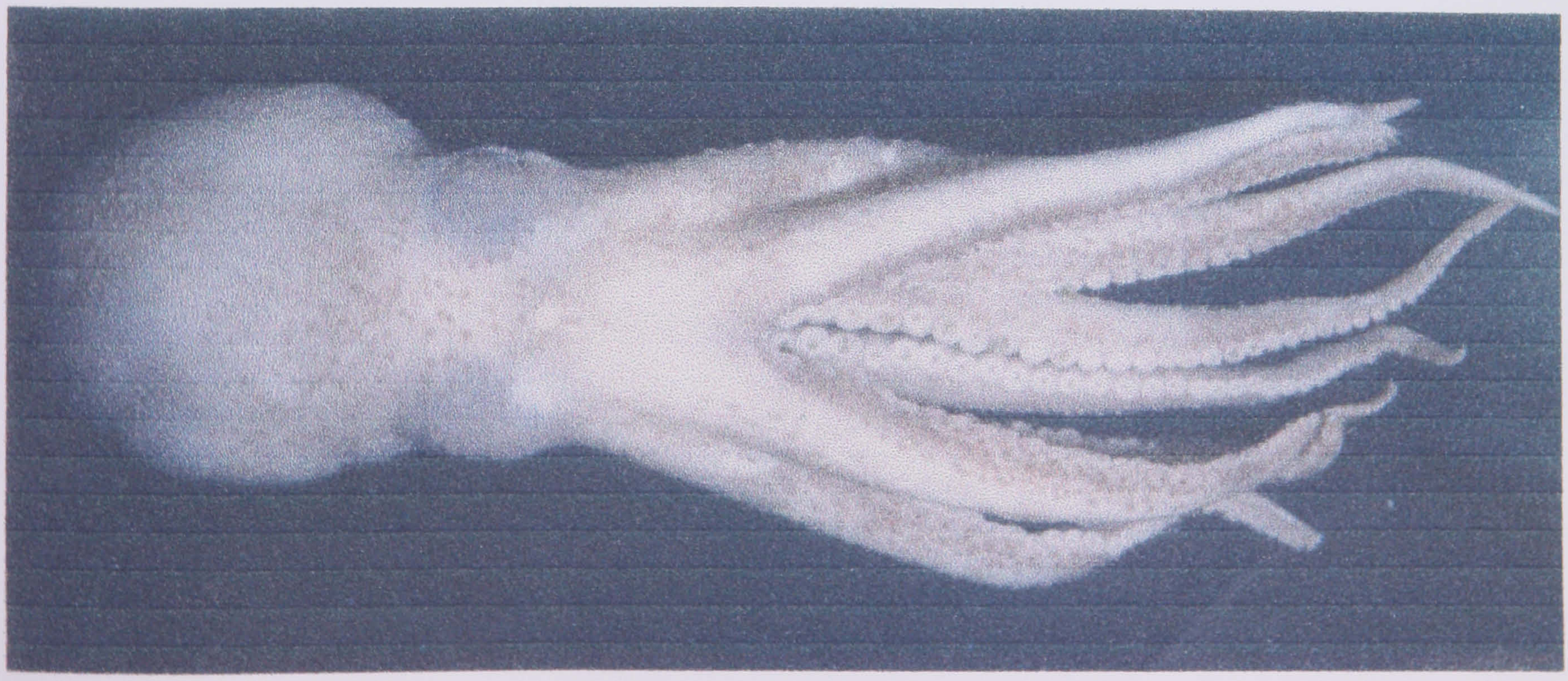


Plate 4.4: *?Graneledone* sp. 25: 71 mm ML.

the genus *Graneledone* partly because of small, but distinctive, hook-like structures on its arms (Plate 4.4); Voss (1976) lists "mantle and arms covered with small to large cartilaginous spiny warts" as a diagnostic feature for this genus. *?Graneledone* sp. 29 (Figure 4.2) was also tentatively assigned to the same genus as the protuberances on the dorsal surface of this species differed markedly from the papillae of the *Pareledone*. They were larger and smoother and bore a resemblance to the 'warts' of *Graneledone antarctica* (Plate 4.6).

Of the remaining twelve non-*Pareledone* species, six were taken from the Antarctic Peninsula. *Benthoctopus ?levis* (Plate 4.5) was the only species captured that had a biserial sucker arrangement. It fits the description of *B. levis* given by Hoyle (1885), differing only in the lengths of arms relative to the mantle (M. Vecchione, pers. comm., 1996). Three species were taken in deep water. *Graneledone antarctica* from 1,500 m is immediately identifiable by its pale 'warts' and its deep purple colouration (Plate 4.6). *Thaumeledone ?brevis* and *Bentheledone ?albida* from 3,200 m (Plate 4.7) are striking because of the lack of colour on the dorsal and ventral surfaces and by their large protruding eyes, both of which are features commonly associated with deep-sea animals. They may be distinguished from one another by the deep purple colouration of the oral surface of the web in *Thaumeledone ?brevis* and by the large, club-like hectocotylus (apparently characteristic of the genus) found in the male of this species. Another species of *Thaumeledone* (sp. 21), assigned to the genus on the basis of hectocotylus shape and the low number of gill lamellae, was taken in moderately deep water (400-1,600 m depth). Its continuous light purple colouration (except on the oral surface of the web which is dark purple) distinguished it from other species of *Thaumeledone* (c.f. absence of chromatophores on dorsal surface in *Thaumeledone ?brevis* (Plate 4.7) and spotted colouration of *Thaumeledone gunteri* (Plate 4.9)). A single specimen of an extremely unusual species (sp. 24) was captured from 600 m. The latter was remarkable in part because of its small size at maturity and its deep purple colouration (Plate 4.8). It is thought that a similar specimen may exist in the Natural

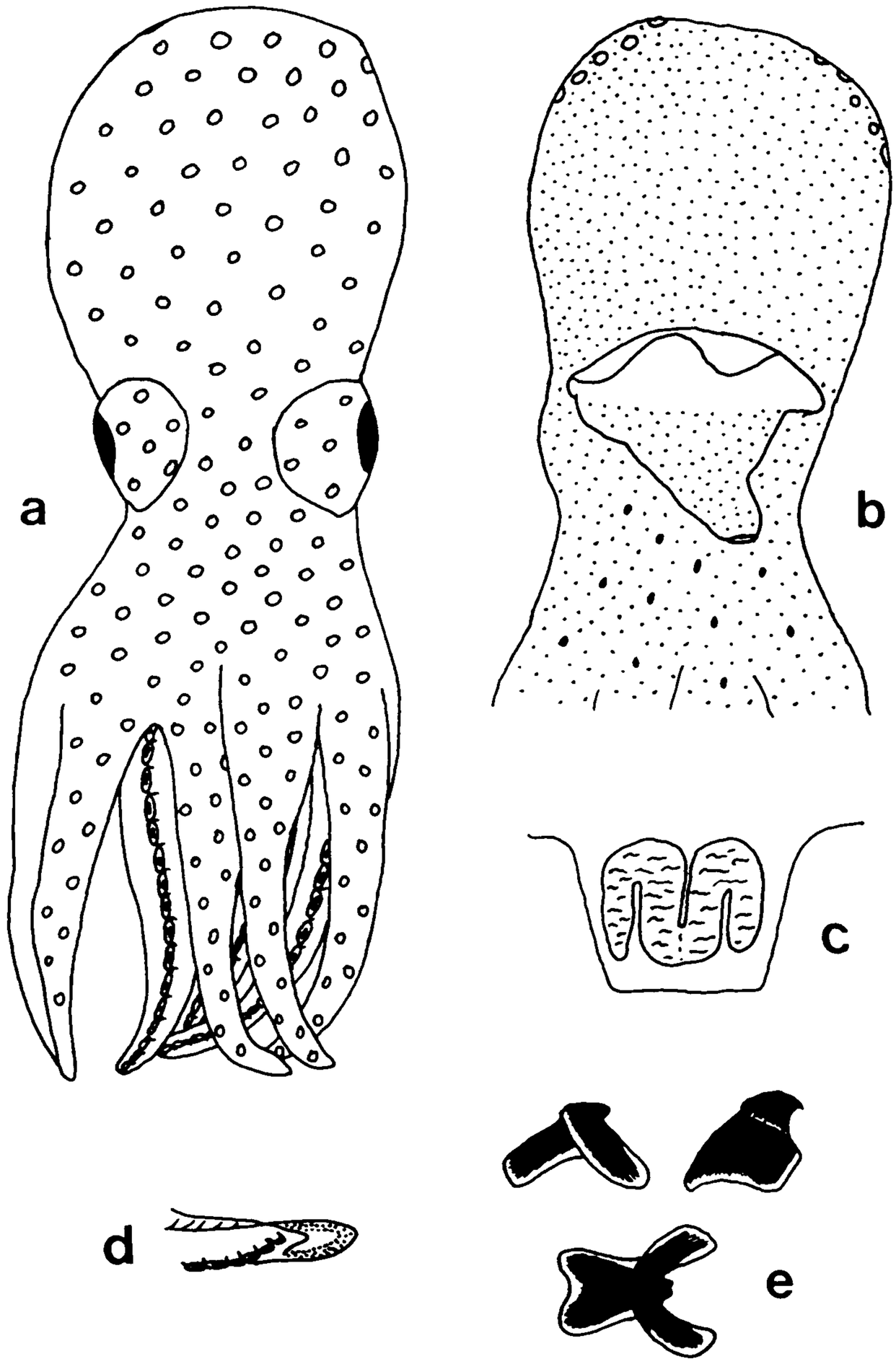


Figure 4.2a-e: *?Graneledone* sp. 29: a, dorsal view of whole animal b, ventral view of mantle and funnel c, funnel organ d, hectocotylus e, beak. All illustrations actual size except beak, twice actual size.

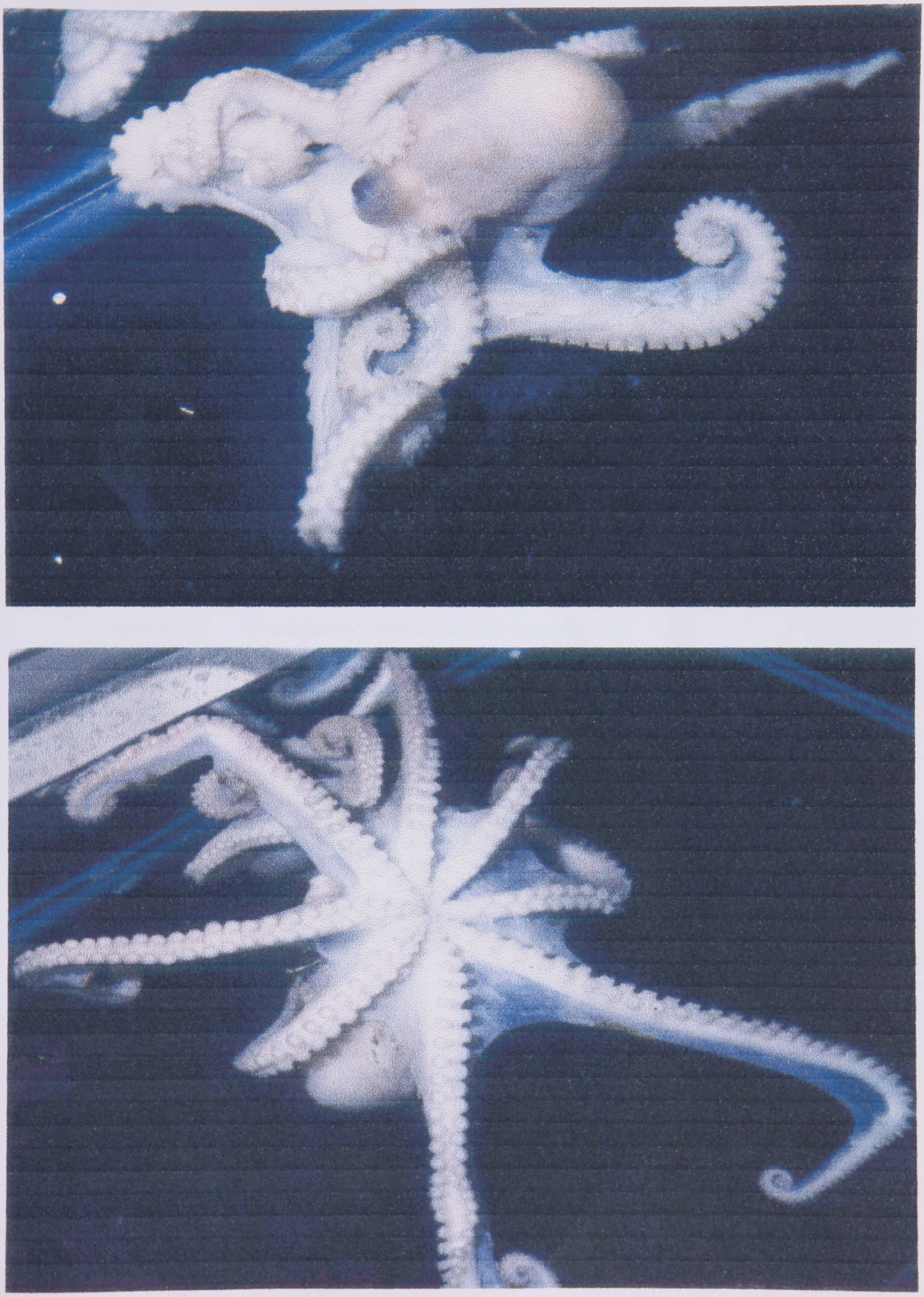


Plate 4.5: *Benthoctopus ?levis*: dorsal view and aboral view.

Plate 4.6: *Graneledone antarctica*: 104 mm ML.

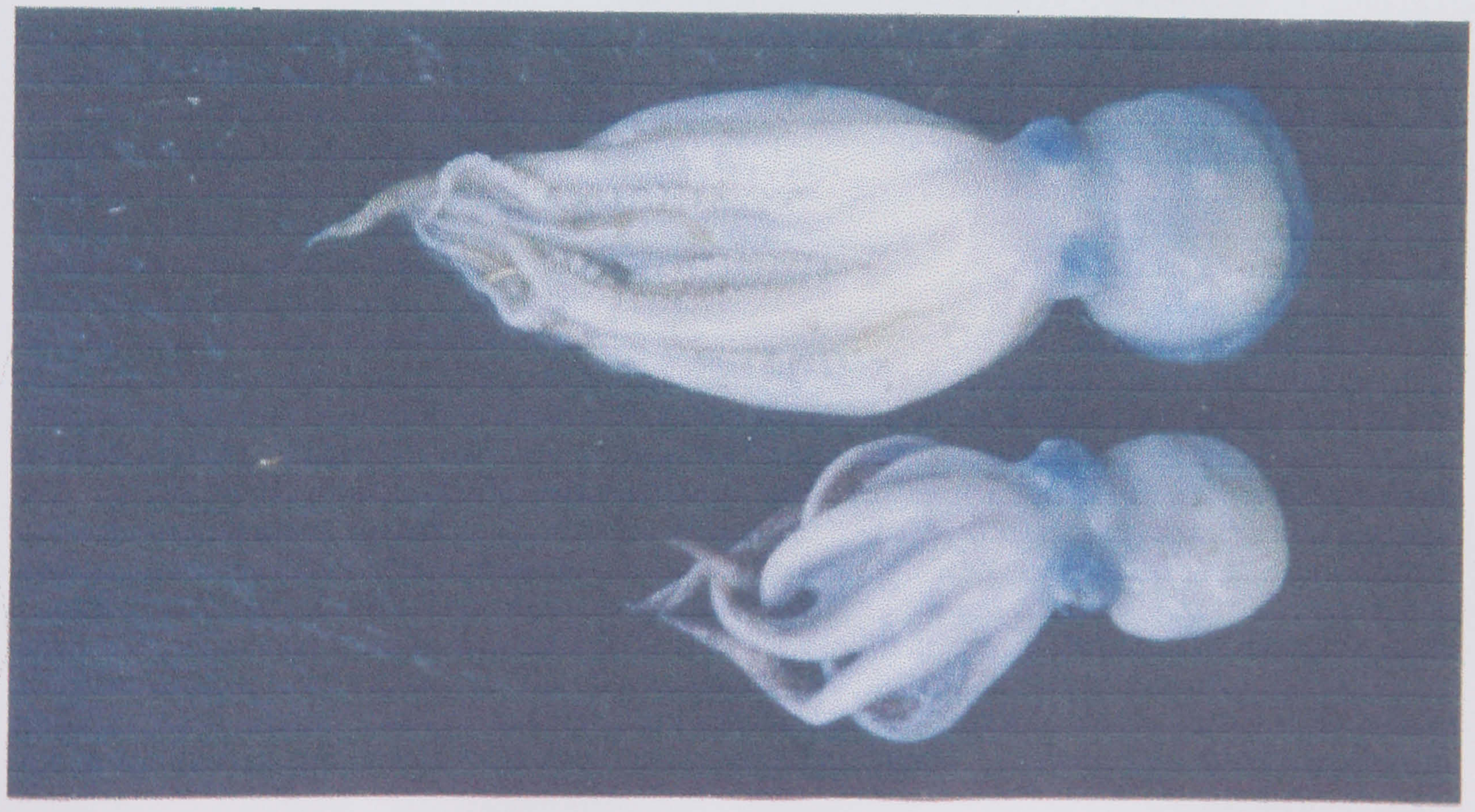


Plate 4.7: *Bentheledone ?albida*: 64 mm ML (top).

Thaumeledone ?brevis: 62 mm ML (bottom).

Plate 4.8: Sp. 24: 23 mm ML.

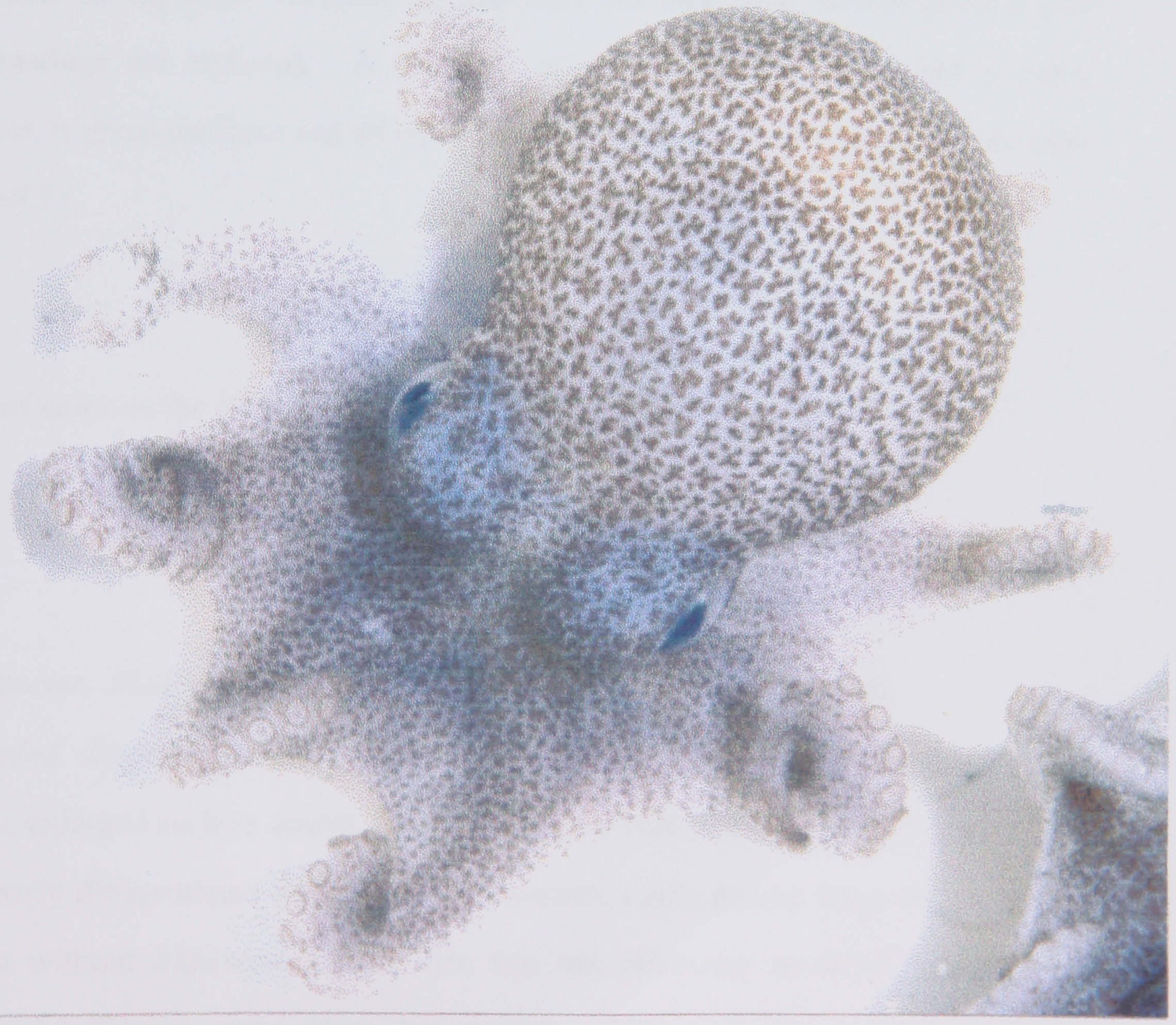


Plate 4.9: *Thaumeledone gunteri*: 50 mm ML.

History Museum in Paris which was found from deep water off New Caledonia (F. G. Hochberg, pers. comm., 1997). These six species are being examined in further detail by Vecchione *et al.*, (in prep).

Three specimens of *Thaumeledone gunteri* were captured from around South Georgia. The species has all the characteristics of the genus (see above), but can be distinguished by its 'spotted' colouration (Plate 4.9). It is currently being redescribed (Stranks *et al.*, in prep).

Of the five species captured from the Falkland Islands, three (*Octopus tehuelchus*, *Benthoctopus eureka* and *Enteroctopus megalocyathus*) are well documented although the genera *Benthoctopus* and *Enteroctopus* are currently under review (M. Vecchione, pers. comm., 1996; F. G. Hochberg, pers. comm., 1997). Two other species (sp. 7 and sp. 8) are apparently undescribed, but as there are only three specimens in total of these two species little progress can be made until further specimens are captured. As these animals were not seen alive, neither photographs nor drawings are included. A selection of counts, measurements and indices, however, is given for these and all other unnamed species not described in detail later (Table 4.1).

Detailed notes on the Pareledone with descriptions of seven new species

Octopodidae

Pareledone Robson, 1932

Type species. Eledone charcoti Joubin, 1905. By subsequent diagnosis.

Ammended diagnosis. Mantle saccular. Arms with small to medium uniserial suckers, enlarged suckers absent, third right arm of males hectocotylised with end of arm clearly differentiated into ligula and calamus, ligula groove long, well marked, shallow without transverse ridges, arm tips not otherwise modified. Web well developed. Funnel organ large VV-shaped. Gills well developed, with 6-11

Table 4.1: Selected counts and indices for species that could not be identified. Sample size refers to numbers from which detailed measurements were taken and does not necessarily reflect numbers caught.

Genus:				*Thaumeledone*			*?Graneledone*	*Pareledone*	*?Graneledone*
Species:	sp. 7	sp. 8	sp. 17	sp. 21	sp. 22	sp. 24	sp. 25	sp. 26	sp. 29
Sample size:	1	2	37	7	13	1	2	1	21
MWI	102.8	111.3	93.1	97.1	96.7	78.3	82.3	91.2	81.1
EDI	34.7	38.2	24.1	26.0	26.0	17.4	25.4	26.5	26.1
FuLI	45.8	51.3	42.3	39.8	44.4	26.1	36.1	47.1	40.4
WDI	36.3	31.5	41.4	44.8	43.7	19.3	24.0	32.8	32.9
MAI	39.6	30.6	60.0	64.9	53.8	40.4	53.4	55.7	67.0
L1	241.7	310.3	158.3	148.5	161.3	230.4	175.3	170.6	140.5
L2	241.7	302.0	151.4	148.6	181.6	247.8	84.5	170.6	145.1
L3	227.8	293.8	156.2	150.1	181.5	243.5	189.1	176.5	146.3
L4	234.7	288.7	159.7	148.1	178.1	230.4	183.9	179.4	140.1
OAI	109.8	106.0	94.8	92.9	80.9	94.6	89.2	95.0	98.4
HSC			27.2	21.5	27.3		31.5	25	23.7
OASC	63	79.5	38.6	30.0	38.8	42	49.5	30	31.9
ASI	6.9	5.7	6.5	4.7	6.7	4.3	5.0	8.8	5.1
LLI			10.8	10.2	9.3		4.4	10.5	9.4
IGL (mode)	7	6	8	5	8	5	9 or 10	8	7
OGL (mode)	7	7	7	4	8	4	9	7	7
SpLI			66.4	17.4	63.8				12.6
SpWI			5.6	3.6	5.7				9.9
EgLI			10.0	7.3		17.4			9.2
EgWI			4.2	4.2		13.0			4.8

lamellae. Ink sac and anal flaps present. Crop well developed without anterior diverticulum. Posterior salivary glands medium sized. Chromatophores absent on dorsal surface of digestive gland. Cartilaginous stylets absent. Spermatophores long and slender. Beak medium sized, rostral tip of lower beak rounded. Radula normal; mulitcuspid rhachidian, lateral teeth and marginal plates well defined. Eggs large (>15 mm).

Pareledone charcoti (Joubin)

Plate 4.10

Eledone charcoti Joubin, 1905: 22, pl 3 figs 1, 2. –Joubin, 1906: 2, pl 1 figs 1, 2. –Joubin, 1914: 35, text figs 1, 2. –Odhner, 1923: 6.

Moschites charcoti. –Hoyle, 1912: 279, text figs 6, 7. –Massy, 1916: 151, text figs 12-21.

Moschites aurorae. Berry, 1917: 20, text figs 14-20, pl 12 fig 9, pl 13 figs 10-12 (see also postscript, 1918).

Graneledone charcoti. –Robson, 1930: 388.

Pareledone charcoti. –Robson, 1932: 270. –Dell, 1959: 93, text figs 4-6. –Roper *et al.*, 1985: 200, 4 figs. –Okutani, 1986: 279, pl 3 figs 25, 26. –Dong, 1991: 183, text fig 1. –Lu and Stranks, 1994: 224, fig 2, text figs 9e-h. –Kubodera and Okutani, 1994: 206, fig 1.

Material examined.

Weddell Sea, PFS *Polarstern*, 5-28 February 1996. —71°03'S 11°25'W, 462 m, Stn 39/001: WS51 (F3, 60 mm ML), WS107 (M3, 55 mm ML). —71°41'S 12°44'W, 227 m, Stn 39/005: WS121 (M3, 53 mm ML), WS149 (F3, 63 mm ML), WS153 (M3, 55 mm ML), WS154 (F3, 76 mm ML), WS156 (M3, 55 mm ML) WS174 (M3, 54 mm ML). —73°23'S 21°11'W, 338 m, Stn 39/011: WS219 (M3, 47 mm ML). —73°18'S 21°10'W, 459 m, Stn 39/012: WS236 (M3, 64 mm ML). —73°42'S 22°31'W, 446 m, Stn 39/015: WS262 (M3, 55 mm ML), WS266 (M3, 52 mm ML), WS281 (M3, 61 mm ML). —73°53'S 22°27'W, 246 m, Stn 39/016: WS308 (M3, 64 mm ML), WS312 (M3, 81 mm ML), WS323 (M3, 58 mm ML), WS323 (F3, 54 mm ML), WS324 (M3, 56 mm ML). —71°32'S 12°26'W, 504 m, Stn 39/029: WS430 (M3, 51 mm ML), WS431 (F3, 75 mm ML).

Antarctic Peninsula, PFS *Polarstern*, 16-23 November 1996. —61°08'S 56°11'W, 380 m, Stn 42/003: AP2 (M3, 52 mm ML), AP34 (F3, 55 mm ML). —61°10'S 56°04'W, 174 m, Stn 42/004: AP78 (M3, 50 mm ML), AP80 (M3, 46 mm ML). —61°15'S 55°52'W, 155 m, Stn 42/008: AP134 (F3, 62 mm ML). —61°16'S 55°56'W, 173 m, Stn 42/009: AP150 (M3, 48 mm ML), AP151 (F3, 50 mm ML), AP154 (F3, 76 mm ML). —61°17'S 54°53'W, 200 m, Stn 42/012: AP324 (M3, 47 mm ML), AP325 (M3, 43 mm ML), AP326 (M3, 51 mm ML), AP328 (M3, 42 mm ML). —61°21'S 54°44'W, 291 m, Stn 42/013: AP357 (F3, 75 mm ML), AP358 (M3, 50 mm ML). —61°14'S

54°38'W, 333 m, Stn 42/014: AP378 (M3, 50 mm ML), AP379 (M3, 46 mm ML), AP380 (M3, 45 mm ML). —61°13'S 54°32'W, 577 m, Stn 42/015: AP425 (M3, 51 mm ML). —61°18'S 55°59'W, 296 m, Stn 42/017: AP495 (F3, 54 mm ML), AP496 (M3, 42 mm ML). —61°30'S 56°15'W, 572 m, Stn 42/019: AP532 (F3, 48 mm ML). —61°19'S 56°33'W, 480 m, Stn 42/021: AP592 (F3, 53 mm ML). —61°14'S 56°26'W, 403 m, Stn 42/022: AP628 (F3, 47 mm ML). —61°06'S 55°59'W, 213 m, Stn 42/023: AP715 (F3, 60 mm ML), AP717 (F3, 60 mm ML). —61°05'S 55°56'W, 174 m, Stn 42/024: AP756 (F3, 54 mm ML). —61°02'S 55°58'W, 313 m, Stn 42/027: AP899 (F3, 55 mm ML). —60°58'S 55°51'W, 198 m, Stn 42/028: AP947 (F3, 56 mm ML), AP948 (F3, 63 mm ML), AP949 (F3, 52 mm ML). —60°50'S 55°42'W, 302 m, Stn 42/029: AP990 (F3, 72 mm ML).

Diagnosis. Arms short. Hectocotylised arm with 25-41 suckers. Gills with 6-8 lamellae per demibranch. Closely set papillae on dorsal surface, one supraocular papilla, papillae absent from ventral surface.

Description. Animals medium sized (ML to 81 mm; TL to 207 mm). Mantle spherical to ovoid (MWI 63.6-87.6-111.5). Eyes medium sized (EDI 13.2-22.6-28.6). Funnel large (FuLI 24.6-38.9-60.0) gently tapered. Arms short (MAI 45.6-60.7-80.0). Arm lengths subequal, arm order usually 4=3=2.1 (ALI L1 114.5-150.0-207.7; L2 109.9-157.3-198.1; L3 117.1-159.6-203.8; L4 106.2-158.0-219.2). Suckers small (ASI 3.6-5.6-8.0). Third right arm of males hectocotylised, shorter than opposite number (OAI 77.8-91.6-108.7). Ligula medium sized (LLI 6.3-8.8-11.8). Calamus distinct. Hectocotylised arm with 25-41 suckers, opposite arm with 34-58 suckers. Web of medium depth (WDI 17.8-30.4-40.3), web formula usually B=C=D.A=E. Gills with 6-8 lamellae per demibranch (mode: inner gill lamellae count 7, outer gill lamellae count 7.) Mature ovarian eggs large, 22 mm x 9 mm (EgLI 13.1-25.0-40.7; EgWI 6.6-11.0-16.7). Spermatophores long (SpLI 96.3-143.6-176.9), slender (SpWI 3.8-5.0-6.7). Males mature at approximately 35-40 mm ML, females at approximately 55-60 mm ML.

Skin sculpture consists of a "pattern of fine, rounded and closely set papillae on dorsal surface; papillae absent from ventral surface. Large unbranched primary papillae present in ocular region, with one supraocular papilla. Ventrolateral integumentary ridge present. In life, colour of resting animals uniformly pink-brown

to purple-brown dorsally, cream-white ventrally. When stimulated, animals become darker in colour, dark purple brown dorsally, cream-white ventrally. Papillae on dorsum usually slightly darker than background, giving spotted appearance. White spots consist of one spot on mid-dorsal brachial crown, and one broad spot on mid-dorsal posterior mantle. White transverse bar present between eyes. Ocelli absent." (Lu and Stranks, 1994).

Taxonomic Summary

Type Specimen. *Eledone charcoti* Joubin, 1905. Submature female, 32 mm ML. In Natural History Museum, Paris. Catalogue number MNHN 5-7-1095.

Type Locality. Antarctic Peninsula. Booth-Wandel Island [65°05'S 63°55'W]. Shore. Expedition Antarctique Française. 3 September, 1904.

Etymology. Named after Dr Charcot, leader of the expedition.

Distribution. Circumpolar. Off Enderby Land [65°56'S 50°52'E], off MacRoberston Land [66°59'-67°40'S 62°49'-65°34'E], Prydz Bay [66°48'-68°26'S 71°25'-78°15E] (Lu and Stranks, 1994); off Queen Mary Land [66°08'S 94°17'E] (Berry, 1917); off Graham Land [65°05'S 63°55'W] (Joubin, 1905); Weddell Sea [71°03'-73°53'S 11°25'-22°31'W], South Shetland Islands [60°50'-61°30'S 54°38'-56°33'W] (this study). Prior to this study the known depth range of the species was 110-683 m (Lu and Stranks, 1994), but specimens were captured at depths of >800 m off the Antarctic Peninsula.

Remarks. Well described by Lu and Stranks, 1994 who made efforts to verify many of the previously caught specimens of this species. The transverse bar between the eyes is often a series of dots and is occasionally absent (Plate 4.10), while more than one spot is sometimes seen on the mid-dorsal brachial crown and on the mid-dorsal posterior mantle.

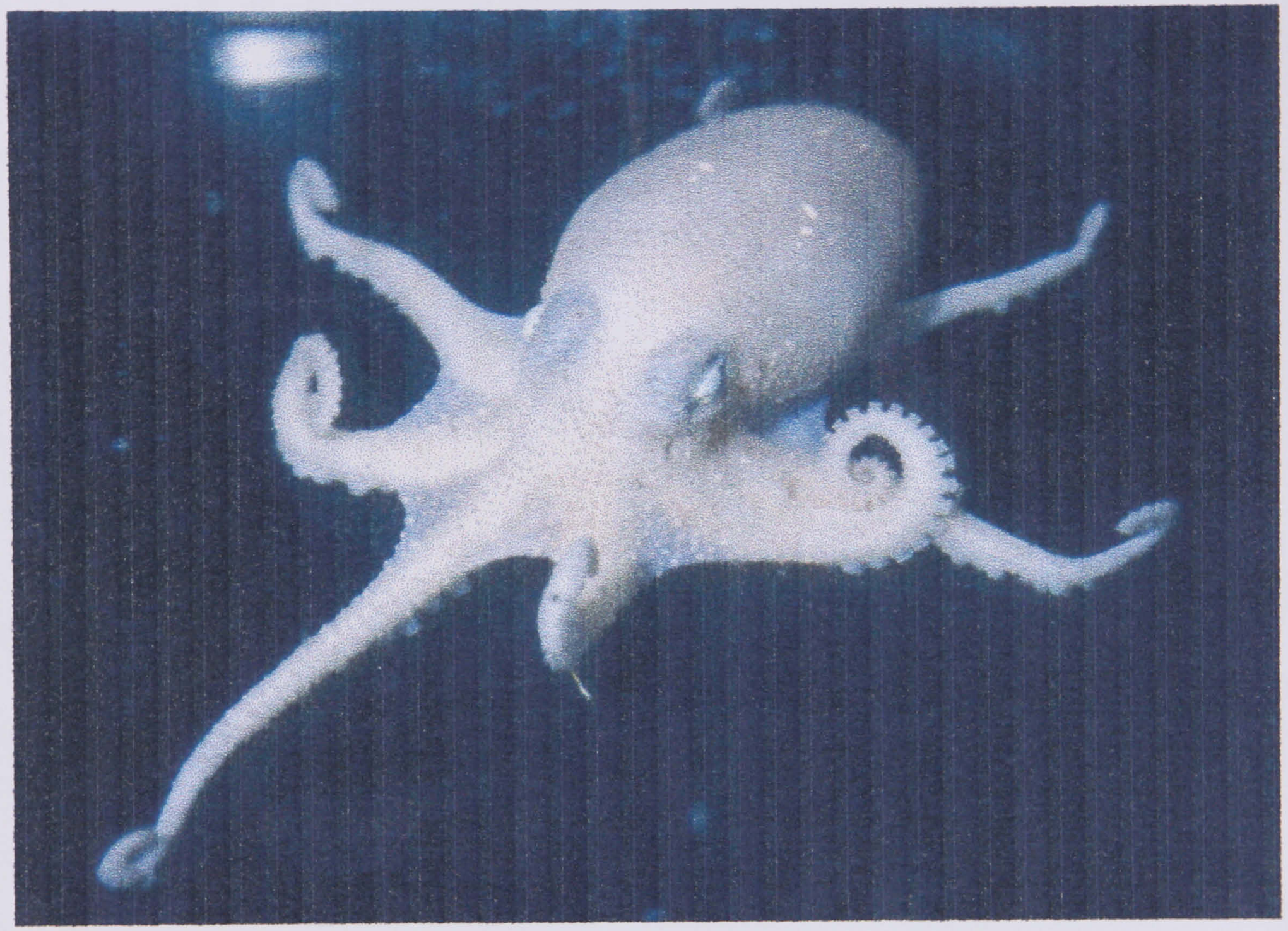


Plate 4.10: *Pareledone charcoti*: 47 mm ML.

Pareledone turqueti (Joubin)

Plate 4.11

Eledone turqueti, Joubin, 1905: 29, pl 3 figs 3-6. –Joubin, 1906: 9, pl 1 figs 3-6. –Joubin, 1914: 37, text figs 3, 4.

Moschites turqueti, –Berry, 1917: 13.

Graneledone turqueti, –Robson, 1930: 390.

Pareledone turqueti, –Robson, 1932: 273, text fig 50. –Roper *et al.*, 1985: 204, 4 figs. –Dong, 1991: 185, text fig 3.

Material examined.

Weddell Sea, PFS *Polarstern*, 5-28 February 1996. —71°03'S 11°25'W, 462 m, Stn 39/001: WS40 (M1, 40 mm ML), WS49 (M2, 61 mm ML), WS50 (M3, 82 mm ML), WS65 (M3, 59 mm ML), WS78 (M3, 73 mm ML). —71°40'S 12°42'W, 254 m, Stn 39/005: WS112 (F2, 91 mm ML). —71°41'S 12°44'W, 227 m, Stn 39/005: WS116 (F1, 51 mm ML), WS119 (F1, 33 mm ML), WS122 (F2, 55 mm ML), WS123 (F2, 56 mm ML), WS135 (M1, 37 mm ML), WS136 (F2, 86 mm ML), WS137 (F2, 82 mm ML), WS138 (F2, 67 mm ML), WS143 (M1, 52 mm ML), WS144 (M1, 35 mm ML), WS 146 (F1, 51 mm ML). —71°19'S 12°17'W, 170 m, Stn 39/002: WS191 (M3, 87 mm ML), WS192 (F2, 74 mm ML). —71°27'S 13°43'W, 212 m, Stn 39/006: WS199 (M2, 51 mm ML). —73°23'S 21°11'W, 338 m, Stn 39/011: WS216 (F1, 33 mm ML), WS217 (F1, 34 mm ML), WS218 (F2, 63 mm ML). —73°18'S 21°10'W, 459 m, Stn 39/012: WS233 (F1, 50 mm ML). —73°36'S 22°19'W, 620 m, Stn 39/013: WS244 (M1, 73 mm ML). —73°42'S 22°31'W, 446 m, Stn 39/015: WS255 (F3, 106 mm ML), WS278 (M3, 90 mm ML), WS280 (M3, 86 mm ML). —73°53'S 22°27'W, 246 m, Stn 39/016: WS287 (M3, 71 mm ML), WS288 (M2, 78 mm ML), WS290 (F3, 100 mm ML), WS291 (M3, 62 mm ML), WS292 (F2, 65 mm ML), WS309 (F2, 66 mm ML), WS310 (F2, 60 mm ML), WS311 (F2, 60 mm ML), WS314 (F2, 66 mm ML), WS315 (F1, 45 mm ML). —73°18'S 21°10'W, 468 m, Stn 39/017: WS336 (F2, 104 mm ML), WS340 (F1, 53 mm ML). —71°32'S 12°26'W, 504 m, Stn 39/029: WS426 (F1, 52 mm ML).

Diagnosis. Arms long. Hectocotylised arm with 31-45 suckers. Gills with 8-10 lamellae per demibranch. Loose, wrinkled, non-gelatinous integument with small, widely scattered papillae on dorsal surface. Enlarged supraocular papillae.

Description. Animals medium sized (ML to 106 mm; TL to 310 mm). Mantle spherical (MWI 70.7-94.4-109.7). Eyes medium sized (EDI 15.4-24.0-31.4). Funnel large (FuLI 26.0-34.0-43.2) gently tapered. Arms long (MAI 34.5-47.4-73.6). Arm

lengths subequal, arm order usually 4=3.2.1 (ALI L1 121.7-188.4-241.2; L2 125.5-195.4-242.4; L3 131.1-205.3-271.2; L4 134.0-207.5-275.0). Suckers small (ASI 5.1-6.8-9.4). Third right arm of males hectocotylised, shorter than opposite number (OAI 79.1-89.6-104.2). Ligula medium sized (LLI 4.0-6.6-8.1). Calamus distinct. Hectocotylised arm with 31-45 suckers, opposite arm with 38-66 suckers. Web of medium depth (WDI 13.2-21.6-31.9), web formula usually C=D.B.A=E. Gills with 8-10 lamellae per demibranch (mode: inner gill lamellae count 9, outer gill lamellae count 9.) Mature ovarian eggs large, 18 mm x 10 mm (EgLI 14.0-15.5-17.0; EgWI 6.0-7.7-9.4). Spermatophores long (SpLI 85.4-121.3-155.2), slender (SpWI 4.0-6.6-8.1). Males mature at approximately 70-80 mm ML, females at approximately 90-100 mm ML.

The integument is often loose and wrinkled, but without the gelatinous consistency reported in *P. harrissoni* or seen in "*P.*" *polymorpha*. The integumental sculpture consists of small, widely scattered papillae on the dorsal surface; the ventral surface is completely smooth. There are often enlarged supraocular papillae but the apparent ability of the animal to contract these papillae so that they lie flat and are undetectable makes it difficult to assess whether these papillae are present in all specimens. The colour of the dorsal surface varies from pale brown to pale purple / grey. There is a faint ventrolateral ridge and chromatophores continue to be dense for 5-10 mm (in a mature animal) beyond this after which they are distributed more sparsely, the ventral surface being creamy in colouration. White integumental markings are present to a greater or lesser extent in all specimens. There appears to be some regional variation with fewest markings seen in specimens from the Antarctic Peninsula (Plate 4.11); these usually consist of small scattered white spots on the dorsal surface of the body and between the eyes. Specimens from South Georgia and the Weddell Sea commonly have a white transverse bar between the eyes as well as markings towards the posterior end of the dorsal mantle, although these markings, especially in the latter region, are highly variable (see also Daly, 1996).

Taxonomic Summary

Type Specimen. *Eledone turqueti* Joubin, 1905. Immature female, 15 mm ML. In Natural History Museum, Paris. Catalogue number not traced.

Type Locality. Antarctic Peninsula. Booth-Wandel Island [65°05'S 63°55'W]. 25 m. Expedition Antarctique Française. 15 March, 1904.

Etymology. Named after Dr Turquet who captured the type specimen.

Distribution. Off Booth Wandel Island [65°05'S] (Joubin, 1905); off South Georgia [53°49'S 35°37'W], Schollaert Channel, Palmer Archipelago (Robson, 1930); off Shag Rocks [52°21'-53°51'S 40°47'-42°44'W], off South Georgia [53°37'-55°26'S 34°44'-39°33'W], Antarctic Peninsula [60°50'-61°21'S 54°38'-55°58'W], Weddell Sea [71°03'-73°53'S 11°26'-22°31'W] (this study). Specimens have also been reported from McMurdo Sound and from as far north as Rio de Janeiro (Massy, 1916). Robson (1932) questioned the identity of these specimens stating they "have been so much damaged I cannot describe them". The Rio specimens are probably either *Eledone massyae* or *E. gaucha* and the conclusion of a recent taxonomy workshop was that *Pareledone turqueti* is probably restricted to western Antarctic waters (see Chapter 1). The recorded depth range of this species is 25-640 m (although the alleged specimens from Brazil were caught in considerably deeper water).

Remarks. This species was originally described from a single immature specimen (TL 42 mm) which had been contracted by the alcohol in which it was preserved. It is probably almost impossible to confirm which of the smooth skinned species of *Pareledone* recognised in this thesis is synonymous with this type specimen, without extracting DNA (if this were possible) from the type specimen. However, the type specimen was taken in shallow water (25 m depth) off the Antarctic Peninsula, and in this study only one of the smooth skinned *Pareledone* species appeared to extend to

the shallowest catches (70 m depth) in this region. Electrophoresis showed that this species was probably the same as those encountered in South Georgia and the Weddell Sea that were similarly identified as *P. turqueti* (Chapter 2). All these specimens satisfied the qualitative description given by Joubin (1905). They differ only slightly from the description given by Lu and Stranks (1994) for *P. harrissoni*, in that the arms are slightly shorter, and the spermatophores are slightly wider. There are also some slight differences in integumental sculpture.

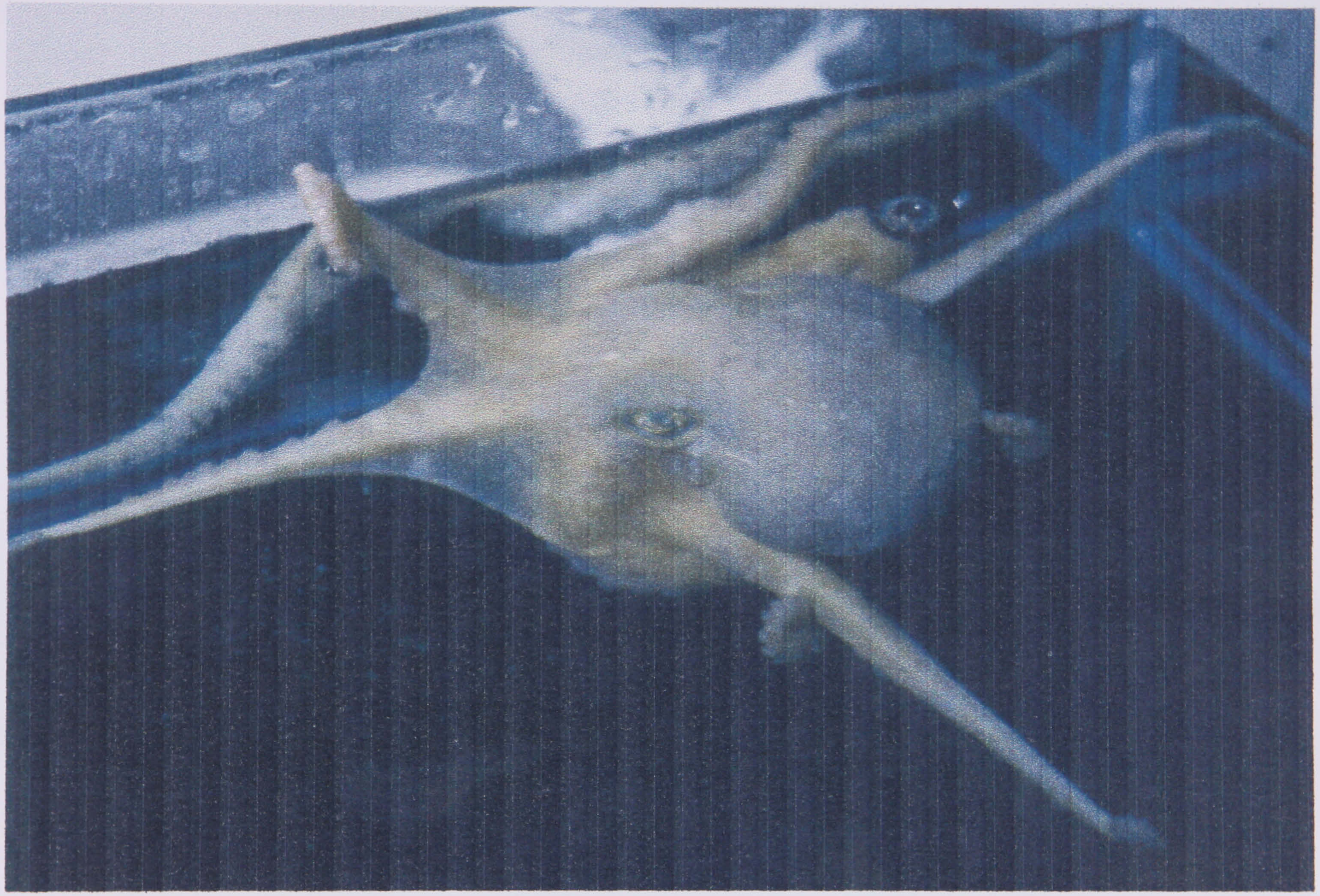


Plate 4.11: *Pareledone turqueti*: 65 mm ML.

Pareledone **sp. 10** sp. nov.

Plate 4.12, Figure 4.3

Material examined.

Holotype: Male to be selected from material below. Catalogue number BMNH1996184 reserved.

Paratype: Female to be selected from material below. Catalogue number BMNH1996185 reserved.

Other material: **Weddell Sea**, PFS *Polarstern*, 5-28 February 1996. —71°40'S 12°42'W, 246 m, Stn 39/005: WS111 (M1, 26 mm ML). —71°41'S 12°44'W, 227 m, Stn 39/005: WS124 (M3, 54 mm ML), WS151 (F2, 55 mm ML), WS152 (M3, 55 mm ML), WS157 (M3, 51 mm ML), WS158 (M2, 46 mm ML), WS159 (M1, 36 mm ML), WS160 (F1, 33 mm ML), WS161 (M1, 29 mm ML), WS162 (M1, 20 mm ML), WS163 (M3, 46 mm ML). —71°32'S 13°35'W, 254 m, Stn 39/006: WS188 (M2, 57 mm ML). —71°27'S 13°43'W, 212 m, Stn 39/006: WS202 (M1, 30 mm ML), WS203 (F1, 31 mm ML), WS204 (F2, 47 mm ML). —73°53'S 22°27'W, 246 m, Stn 39/016: WS289 (M3, 55 mm ML), WS313 (M3, 49 mm ML), WS317 (F1, 36 mm ML), WS319 (M3, 53 mm ML), WS320 (M3, 50 mm ML), WS321 (F2, 55 mm ML), WS326 (M3, 62 mm ML), WS327 (F2, 64 mm ML), WS328 (M3, 51 mm ML), WS329 (M3, 56 mm ML), WS331 (M1, 33 mm ML).

Diagnosis. Arms medium to long. Hectocotylised arm with 29-38 suckers. Gills with 6-8 lamellae per demibranch. Closely set, rounded papillae on dorsal surface. Large supraocular papilla and one enlarged papilla on the posterior dorsal mantle.

Description. Animals medium sized (ML to 64 mm; TL to 189 mm). Mantle spherical to ovoid (MWI 64.9-88.9-100.0) (Figure 4.3a). Eyes medium sized (EDI 17.5-24.6-35.0). Funnel large (Figure 4.3b) (FuLI 31.9-37.6-50.0) gently tapered (Figure 4.3c). Arms medium to long (MAI 42.6-49.1-54.0). Arm lengths subequal, arm order usually 4=3.2=1 (ALI L1 133.3-181.7-213.9; L2 167.3-183.7-219.4; L3 172.7-194.5-225.0; L4 176.4-197.4-230.6). Suckers small (ASI 3.4-5.5-8.5). Third right arm of males hectocotylised, shorter than opposite number (OAI 66.0-78.9-92.8). Ligula medium sized (LLI 8.8-10.0-11.3). Calamus distinct (Figure 4.3d). Hectocotylised arm with 29-38 suckers, opposite arm with 45-56 suckers. Web of medium depth (WDI 17.1-22.4-28.1), web formula usually C=D.B.A.E. Gills with 6-8 lamellae per demibranch (mode: inner gill lamellae count 7, outer gill lamellae

count 7.) No fully mature females were encountered. Spermatophores long (SpLI 98.0-128.7-145.1), slender (SpWI 3.6-4.2-4.6) (Figures 4.3f, g, j). Males mature at approximately 45-50 mm ML.

The integumental sculpture consists of closely set, rounded papillae on the dorsal surface. Large supraocular papillae are present as well as an enlarged papilla at the posterior end of the dorsal mantle. The ventral surface is completely smooth; papillae cease at the ventrolateral integumentary ridge. Dense chromatophores on the dorsal surface give rise to a pale to golden brown colouration. There is a peppering of brown chromatophores directly adjacent to the ventrolateral ridge but the ventral surface is predominantly creamy white in colouration. White spots may be present on the dorsal brachial crown and between the eyes (often as a white transverse bar). A white bar is present in the midline just anterior to the enlarged papilla on the posterior dorsal mantle (Figure 4.3a).

Distribution. Weddell Sea [71°03'-73°53'S 11°26'-22°27'W] (this study). The recorded depth range of this species is 212-481 m.

Remarks. Of the eight species of *Pareledone* with dense arrangements of papillae *P.* sp. 10 is unique because of its pattern of enlarged papillae. Enlarged papillae are also characteristic of *P. framensis* and *P.* sp. 19. *P.* sp. 10 is distinguished from *P. framensis* as in the former species papillae are restricted to the dorsal surface. *P.* sp. 10 is distinguished from *P.* sp. 19 by the differing arrangement of white markings in the two species.

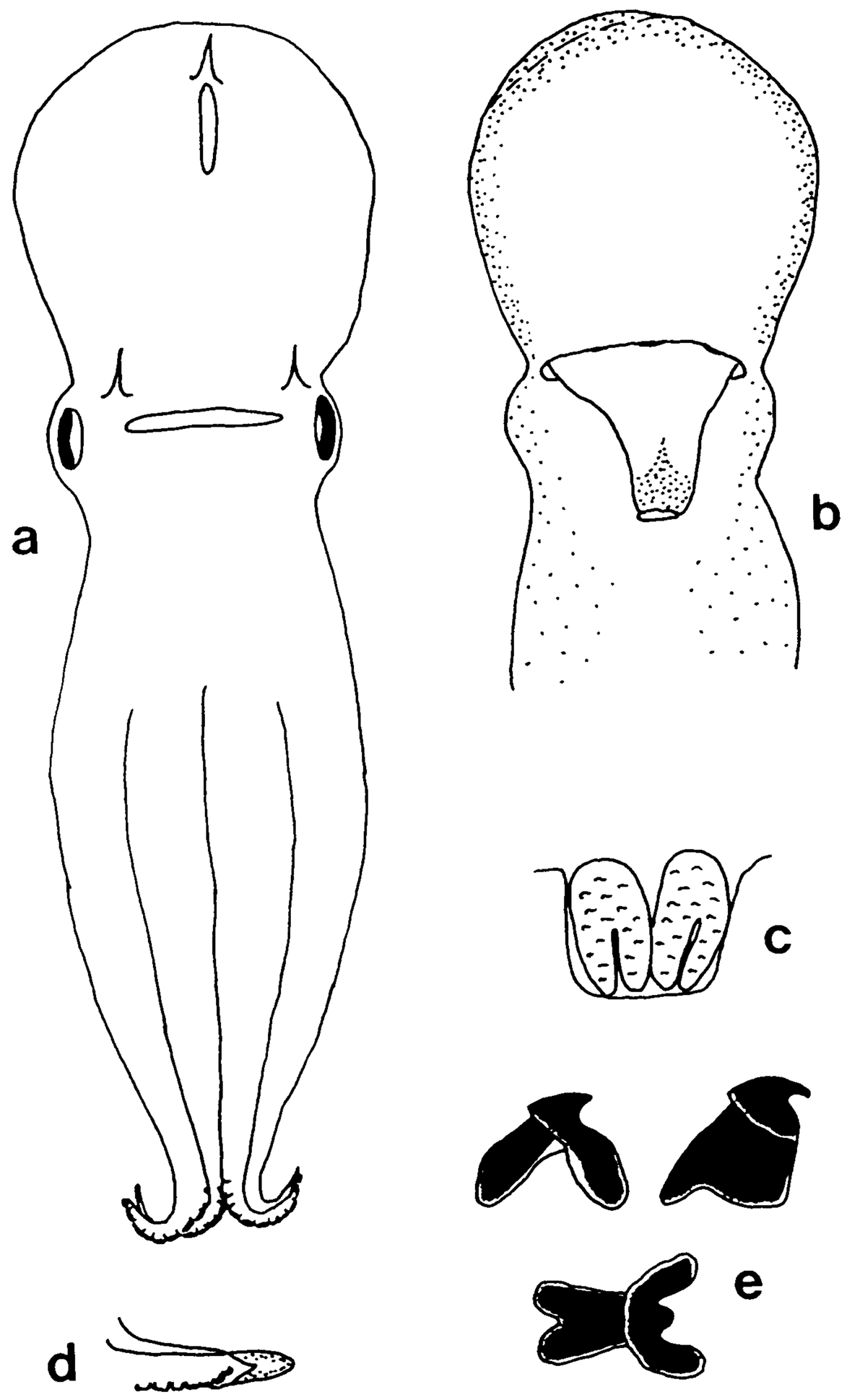


Figure 4.3a-e: *Pareledone* sp. 10 sp. nov.: a. dorsal view of whole animal b, ventral view of mantle and funnel c, funnel organ d, hectocotylus e, beak. All illustrations actual size except beak, twice actual size.

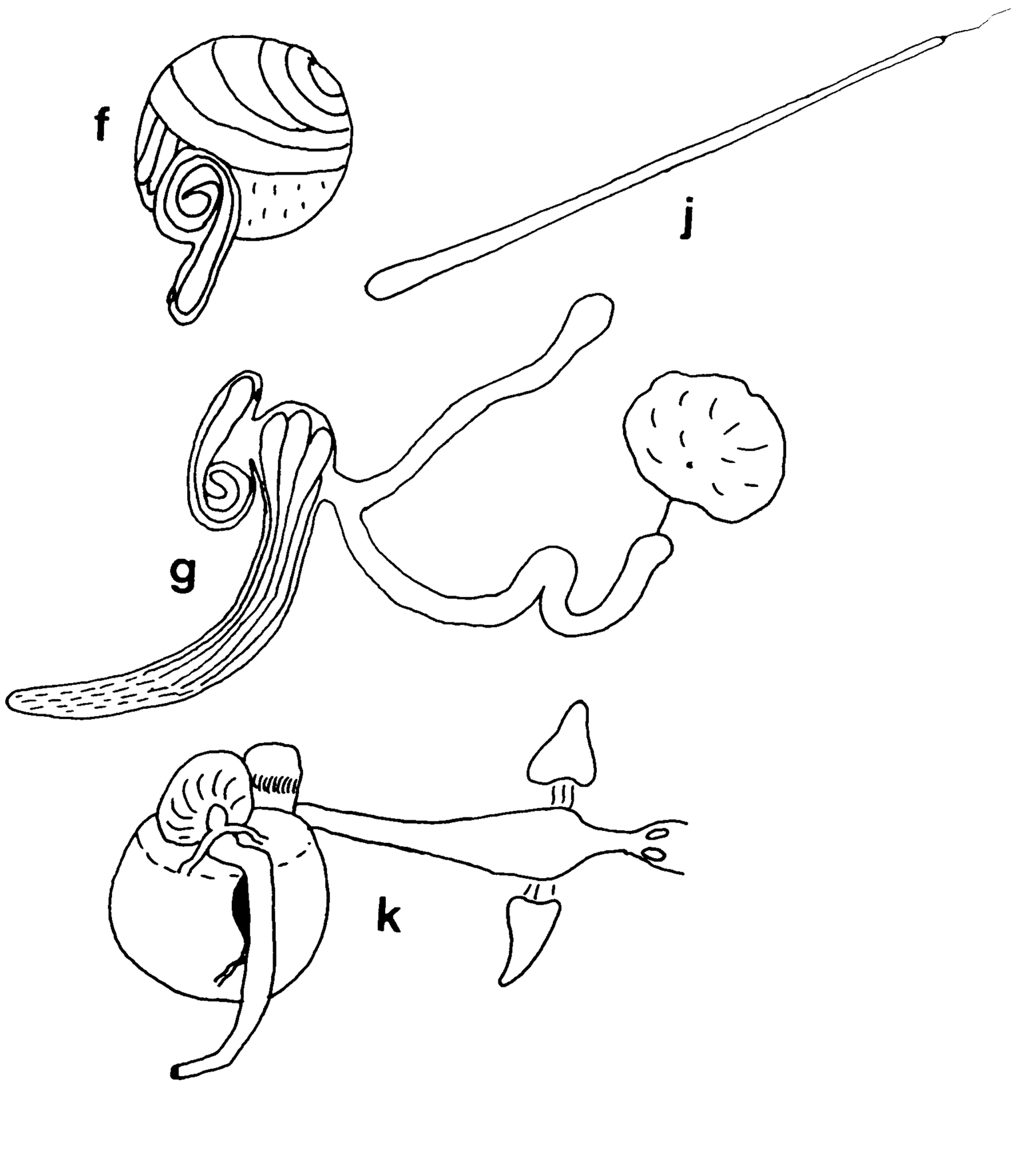


Figure 4.3f-k: *Pareledone* sp. 10 sp. nov.: f, male reproductive system as positioned in situ g, male reproductive organs dissected out j, mature spermatophore k, digestive system. All illustrations actual size.

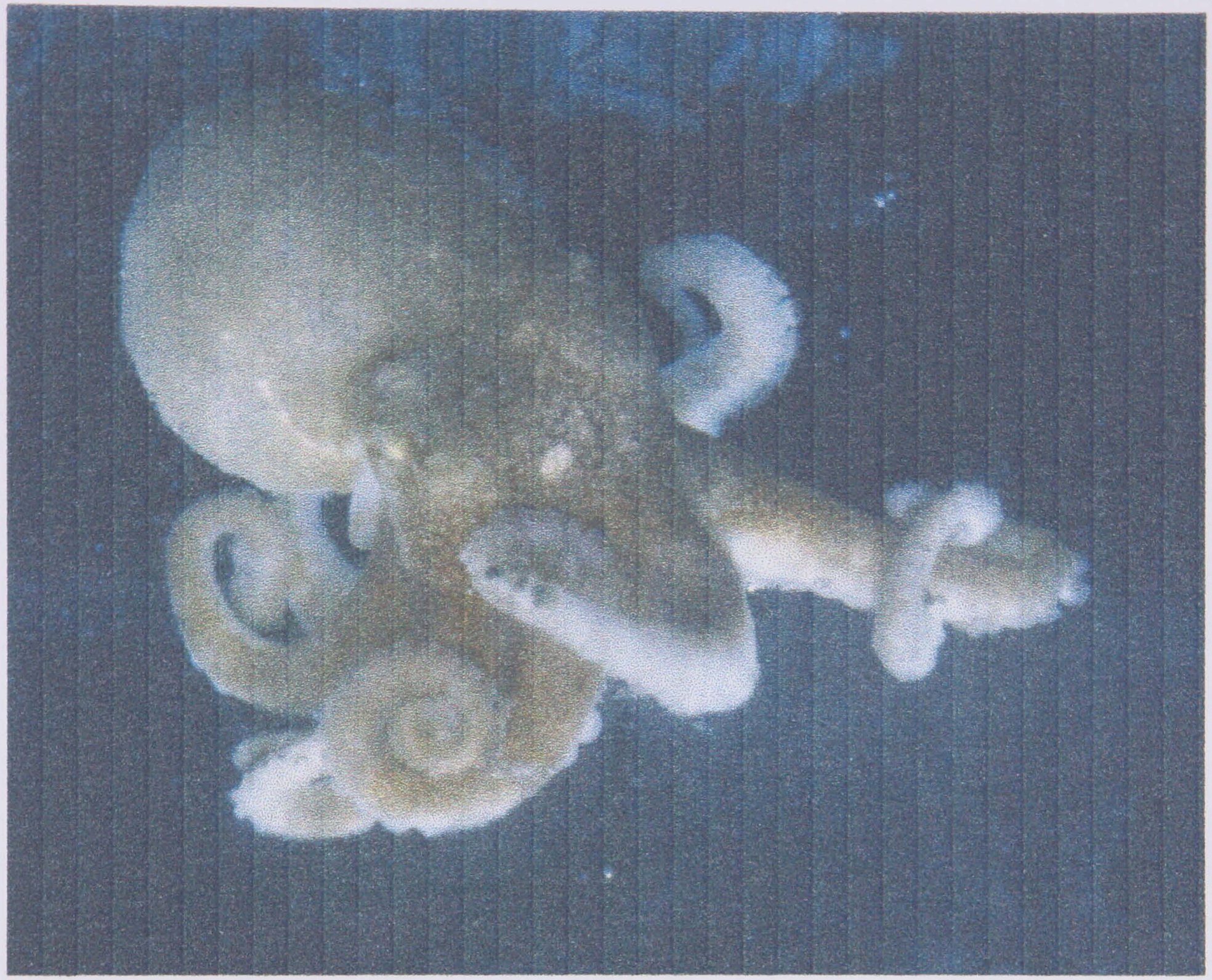


Plate 4.12: *Pareledone* sp. 10 sp. nov.: 47 mm ML.

Pareledone sp. 11 sp. nov.

Plate 4.13, Figure 4.4

Material examined.

Holotype: Male to be selected from material below. Catalogue number BMNH1996186 reserved.

Paratype: Female to be selected from material below. Catalogue number BMNH1996187 reserved.

Other material: **Weddell Sea**, PFS *Polarstern*, 5-28 February 1996. —71°03'S 11°25'W, 462 m, Stn 39/001: WS55 (F1, 27 mm ML). —73°18'S 21°10'W, 459 m, Stn 39/012: WS235 (M3, 41 mm ML), WS237 (F1, 32 mm ML). —73°42'S 22°31'W, 446 m, Stn 39/015: WS256 (F1, 30 mm ML), WS257 (M2, 36 mm ML), WS258 (F2, 48 mm ML), WS259 (F1, 28 mm ML), WS260 (M1, 25 mm ML). —71°32'S 12°26'W, 504 m, Stn 39/029: WS373 (M3, 44 mm ML), WS375 (F2, 40 mm ML), WS376 (F1, 38 mm ML), WS382 (M3, 38 mm ML), WS383 (M3, 43 mm ML), WS415 (M3, 44 mm ML), WS417 (M3, 40 mm ML), WS422 (M3, 42 mm ML), WS423 (M3, 45 mm ML), WS424 (F2, 43 mm ML), WS427 (M3, 46 mm ML).

Diagnosis. Arms medium length. Hectocotylised arm with 29-33 suckers. Gills with 6-8 lamellae per demibranch. Small, rounded, closely set papillae on dorsal and ventral surfaces with very small supraocular papillae. Irridescent transverse bar between the eyes.

Description. Small animals (ML to 48 mm ML; TL to 134 mm ML). Mantle spherical (MWI 85.2-97.8-114.6) (Figure 4.4a). Eyes medium sized (EDI 22.5-28.5-35.0). Funnel large (Figure 4.4b) (FuLI 33.3-39.0-45.5) gently tapered (Figure 4.4c). Arms medium length (MAI 44.1-55.6-61.2). Arm lengths approximately equal (ALI L1 143.2-166.2-196.7; L2131.0-169.1-203.3; L3 156.8-173.1-203.3; L4 138.6-171.5-226.7). Suckers small (ASI 2.3-4.4-6.7). Third right arm of males hectocotylised, shorter than opposite number (OAI 71.2-82.4-96.9). Ligula medium sized (LLI 7.0-10.7-13.3). Calamus distinct (Figure 4.4d). Hectocotylised arm with 29-33 suckers, opposite arm with 35-49 suckers. Web of medium depth (WDI 21.5-26.5-31.1), web formula usually A.B.C.D.E. Gills with 6-8 lamellae per demibranch (mode: inner gill lamellae count 7, outer gill lamellae count 7.) No fully mature females were

encountered. Spermatophores long (SpLI 110.9-125.3-148.8), slender (SpWI 4.7-5.6-6.0) (Figure 4.4f, g, j). Males mature at approximately 35-40 mm ML.

The integumental sculpture consists of small, rounded, closely set papillae on the dorsal and ventral surfaces with very small supraocular papillae. There is a faint ventrolateral integumentary ridge. Dense chromatophores give rise to a purple / grey colouration on all surfaces, including the oral surface of the web. White markings consist of a single transverse bar between the eyes which is iridescent when wet.

Distribution. Weddell Sea [71°03'-73°42'S 11°26'-22°31'W] (this study). The recorded depth range of this species is 354-571 m.

Remarks. This species differs from all eight species of *Pareledone* with dense arrangements of papillae except *P. framensis* in that papillae continue onto the ventral surface. It differs from *P. framensis* in size, colour and in the absence of enlarged papillae.

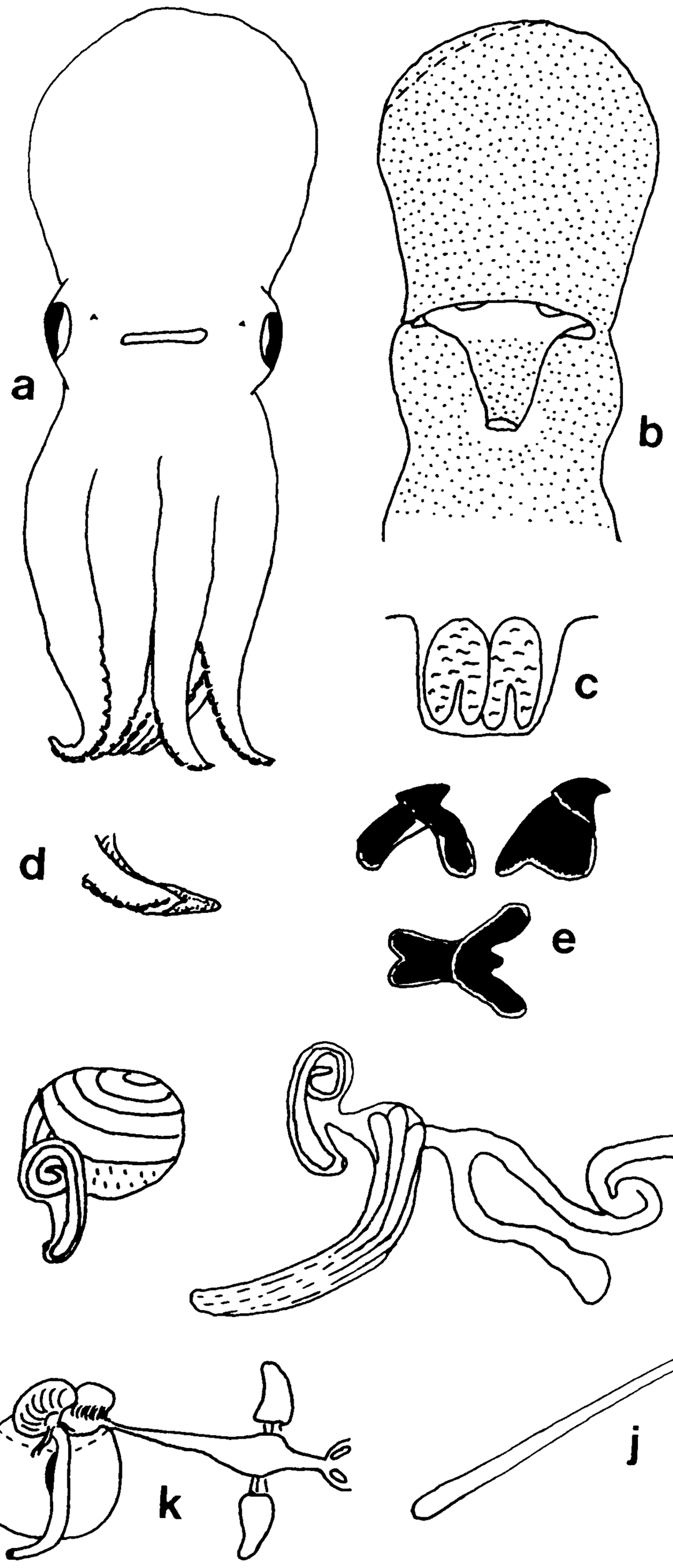


Figure 4.4a-k: *Pareledone* sp. 11 sp. nov.: a, dorsal view of whole animal b, ventral view of mantle and funnel c, funnel organ d, hectocotylus e, beak f, male reproductive system as positioned in situ g, male reproductive organs dissected out j, mature spermatophore k, digestive system. All illustrations actual size except beak, twice actual size.

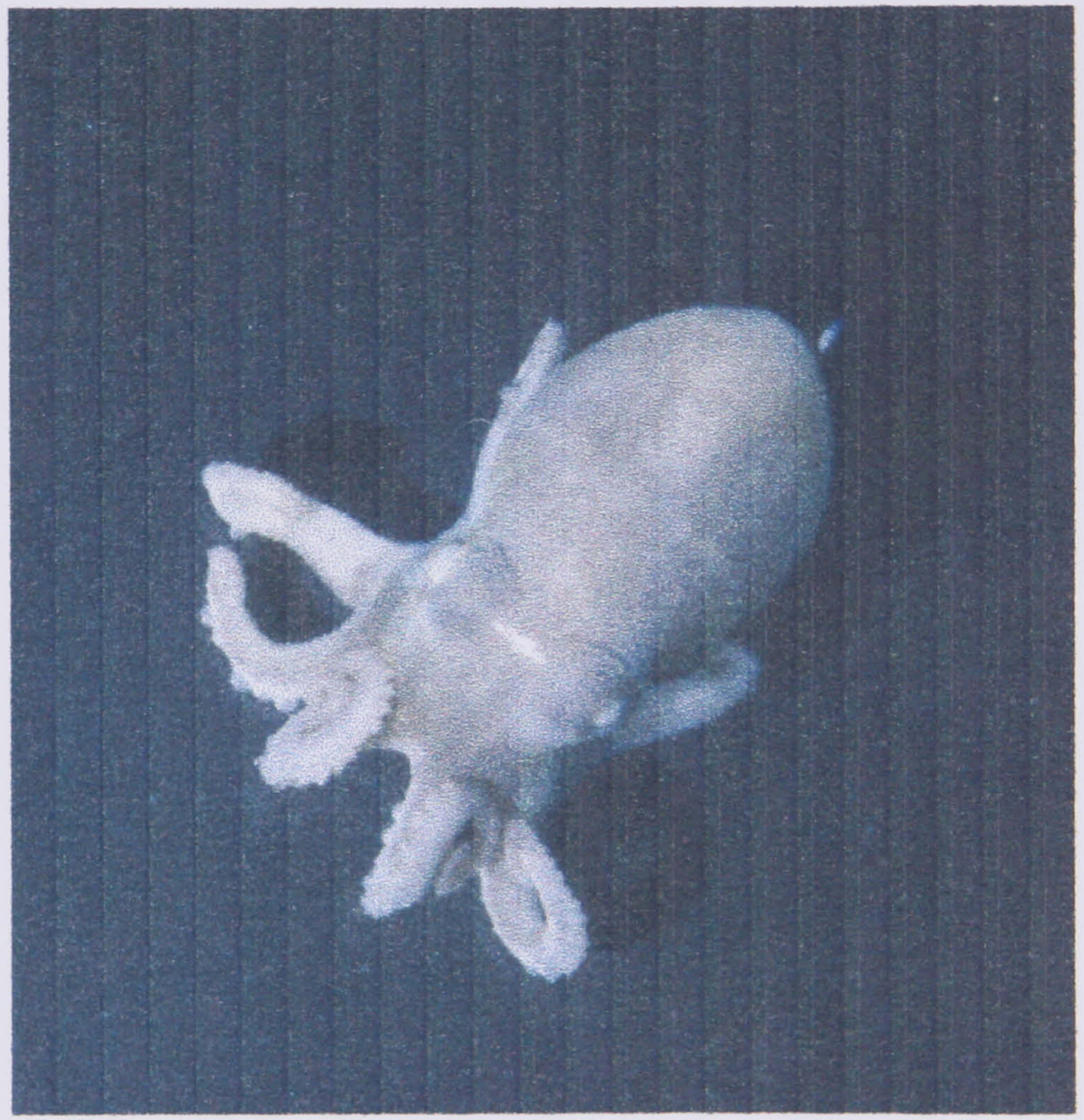


Plate 4.13: *Pareledone* sp. 11 sp. nov.: 46 mm ML.

***Pareledone* sp. 13** sp. nov.

Plate 4.14, Figure 4.5

Material examined.

Holotype: Male to be selected from material below. Catalogue number BMNH1996188 reserved.

Paratype: Female to be selected from material below. Catalogue number BMNH1996189 reserved.

Other Material: **Weddell Sea**, PFS *Polarstern*, 5-28 February 1996. —71°34'S 12°26'W, 604 m, Stn 39/009: WS197 (M1, 74 mm ML). —73°18'S 21°10'W, 459 m, Stn 39/012: WS234 (F1, 58 mm ML), WS238 (F3, 108 mm ML). —73°36'S 22°19'W, 620 m, Stn 39/013: WS243 (F1, 64 mm ML), WS245 (M1, 78 mm ML). —73°36'S 22°36'W, 850 m, Stn 39/014: WS249 (F1, 55 mm ML), WS252 (F2, 100 mm ML). —73°42'S 22°31'W, 446 m, Stn 39/015: WS274 (M1, 28 mm ML), WS275 (M1, 28 mm ML), WS276 (F1, 36 mm ML), WS277 (M1, 36 mm ML), WS283 (M1, 51 mm ML). —73°18'S 21°10'W, 468 m, Stn 39/017: WS335 (M1, 52 mm ML), WS337 (M1, 49 mm ML), WS33 (F1, 53 mm ML). —71°32'S 12°26'W, 504 m, Stn 39/029: WS366 (M2, 62 mm ML), WS367 (M1, 44 mm ML), WS369 (M1, 55 mm ML), WS370 (M2, 81 mm ML), WS371 (F1, 46 mm ML), WS377 (F2, 75 mm ML), WS379 (F1, 40 mm ML), WS380 (F1, 34 mm ML), WS428 (F1, 47 mm ML).

Diagnosis. Arms medium length. Hectocotylised arm with 34-40 suckers. Gills with 8-11 lamellae per demibranch. Finely scattered papillae on dorsal surface. No supraocular papillae. Ventral surface smooth.

Description. Animals medium sized (ML to 108 mm; TL to 295 mm). Mantle spherical (MWI 85.5-96.8-108.5) (Figure 4.5a). Eyes medium sized (EDI 18.8-27.0-35.7). Funnel large (Figure 4.5b) (FuLI 28.3-34.5-42.9) gently tapered (Figure 4.5c). Arms medium length (MAI 41.0-52.6-80.0). Arm lengths subequal, arm order usually 3=4.2.1 (ALI L1 110.0-168.1-210.3; L2 113.0-177.2-220.6; L3 125.0-186.8-244.1; L4 119.0-185.8-238.2). Suckers medium sized (ASI 5.9-7.6-10.7). Third right arm of males hectocotylised, shorter than opposite number (OAI 78.1-92.3-107.2). Ligula medium sized (LLI 1.8-7.0-9.8). Calamus distinct (Figure 4.5d). Hectocotylised arm with 34-40 suckers; opposite arm with 42-55 suckers. Web of medium depth (WDI 19.8-26.0-32.0), web formula usually C=D.B.A=E. Gills with 8-11 lamellae per demibranch (mode: inner gill lamellae count 9, outer gill lamellae

count 9.) Only one fully mature female was encountered. Mature ovarian eggs large, 12 mm x 7 mm (EgLI 11.1; EgWI 6.5) (Figures 4.5h, i). No fully mature males were encountered. Females mature at approximately 100-110 mm ML.

The integumental sculpture consists of finely scattered papillae on the dorsal surface. There are no supraocular papillae. The ventral surface is completely smooth and is separated from the dorsal surface by a fine ventro-lateral ridge. Both dorsal and ventral surfaces are densely covered in chromatophores giving rise to a red brown colouration, as is the oral surface of the web. White markings are, however, associated with the scattered papillae, giving the dorsal surface a speckled appearance (Plate 4.14). Additional white markings may be seen as a transverse bar between the eyes and a marking on the posterior dorsal mantle (Figure 4.5a).

Distribution. Weddell Sea [71°03'-73°42'S 11°26'-22°36'W], Antarctic Peninsula [60°50'-62°19'S 54°32'-59°13'W] (this study). The recorded depth range of this species is 222-889 m.

Remarks. This species is very similar to the other smooth skinned *Pareledone* but its validity has been confirmed by electrophoresis. It has shorter arms and a relatively longer hectocotylus than *P. harrissoni*. It differs in integumental sculpture from *P. turqueti*, and also has slightly shorter arms with larger suckers. It differs from those species with dense papillae by the higher number of gill lamellae, as do all the smoother skinned species.

Specimens of greater maturity were later found from the Antarctic Peninsula. Measurements from these specimens allowed the following indices to be calculated: mean SpLI, 114.3; mean SpWI, 4.8; mean EgLI, 12.9; mean EgWI, 7.2; maximum egg dimensions, 18 mm x 11 mm.

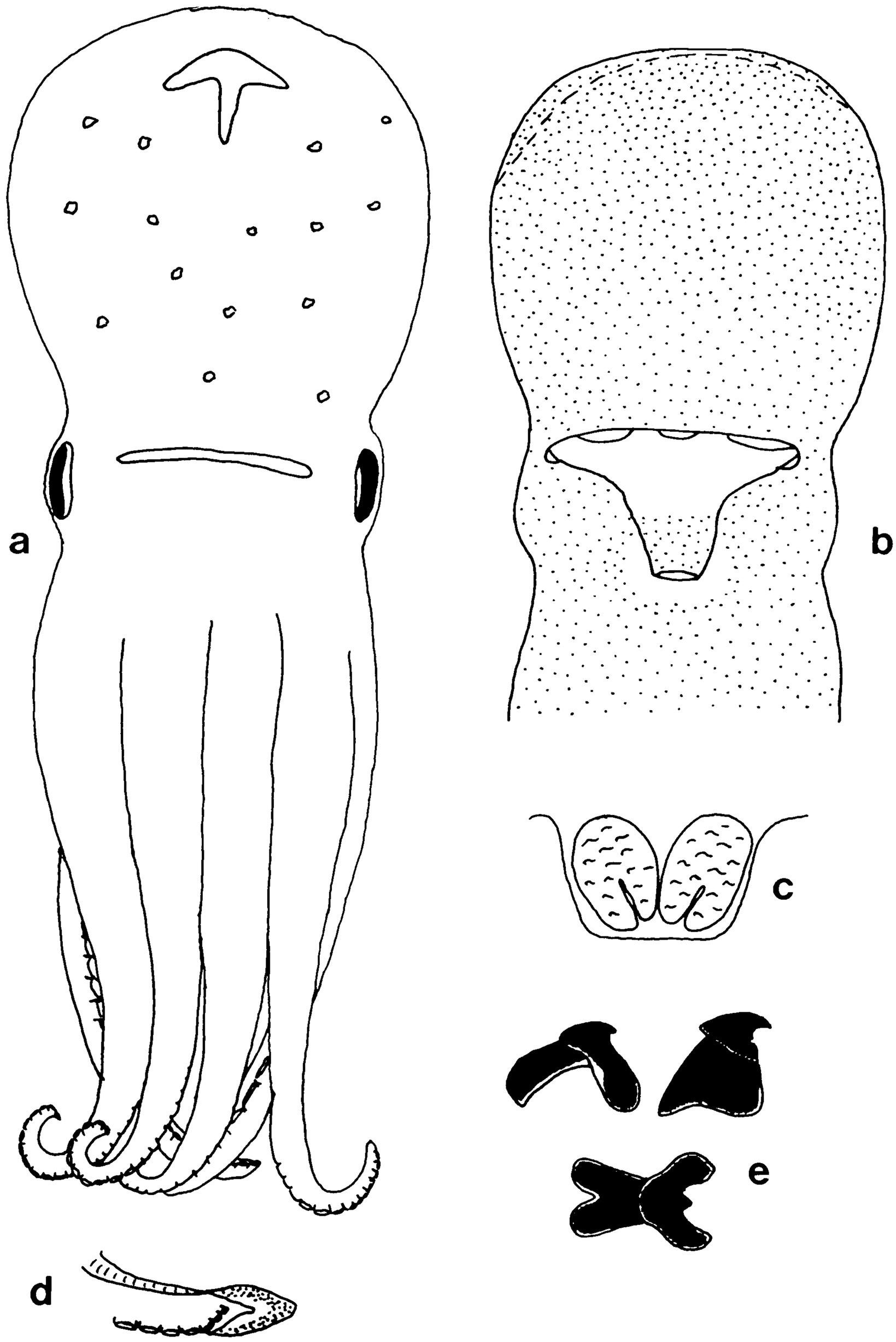


Figure 4.5a-e: *Pareledone* sp. 13 sp. nov.: a, dorsal view of whole animal b, ventral view of mantle and funnel c, funnel organ d, hectocotylus e, beak. All illustrations actual size including beak.

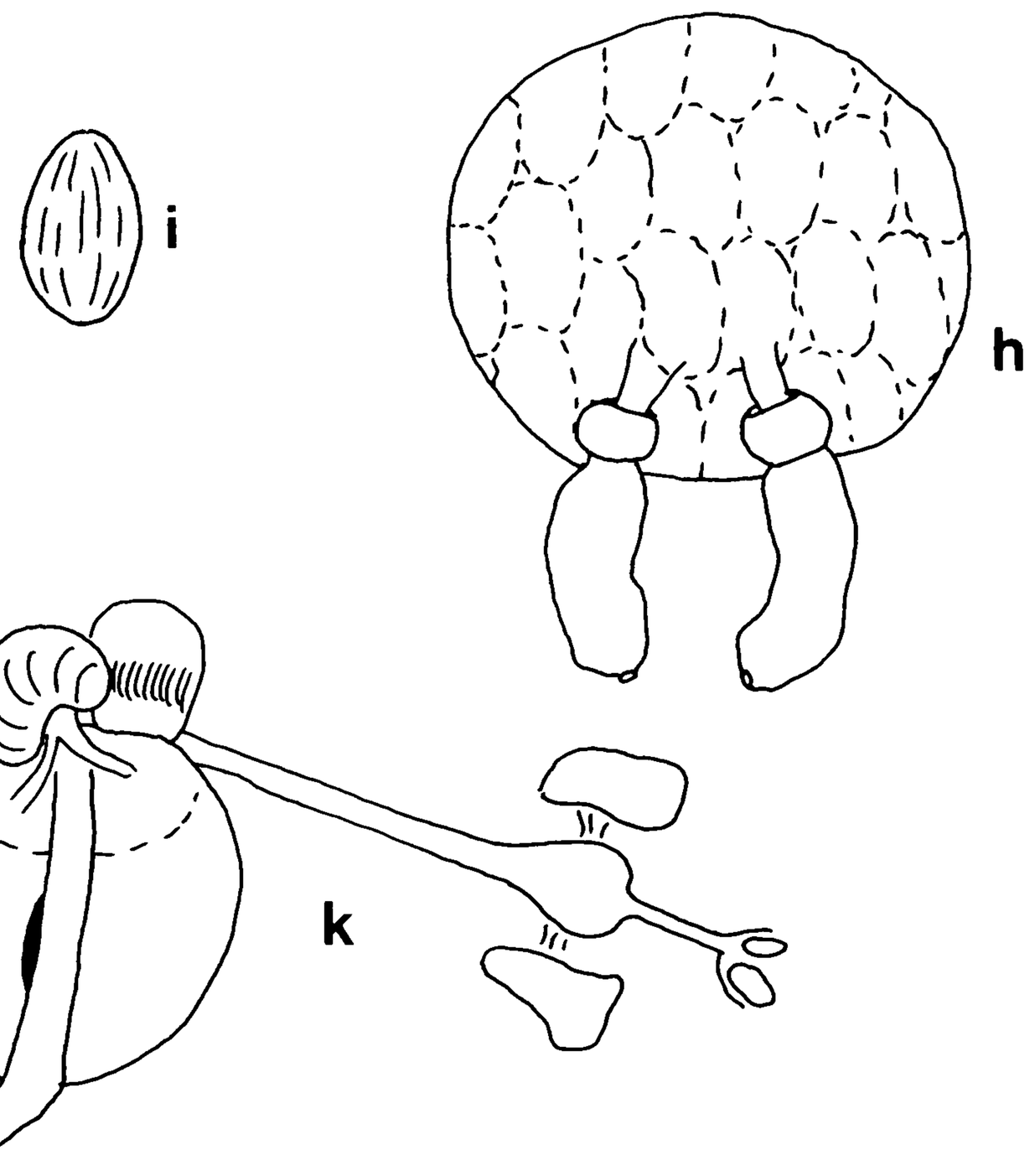


Figure 4.5h-k: *Pareledone* sp. 13 sp. nov.: h, female reproductive system i, mature ovarian egg k, digestive system. All illustrations actual size.

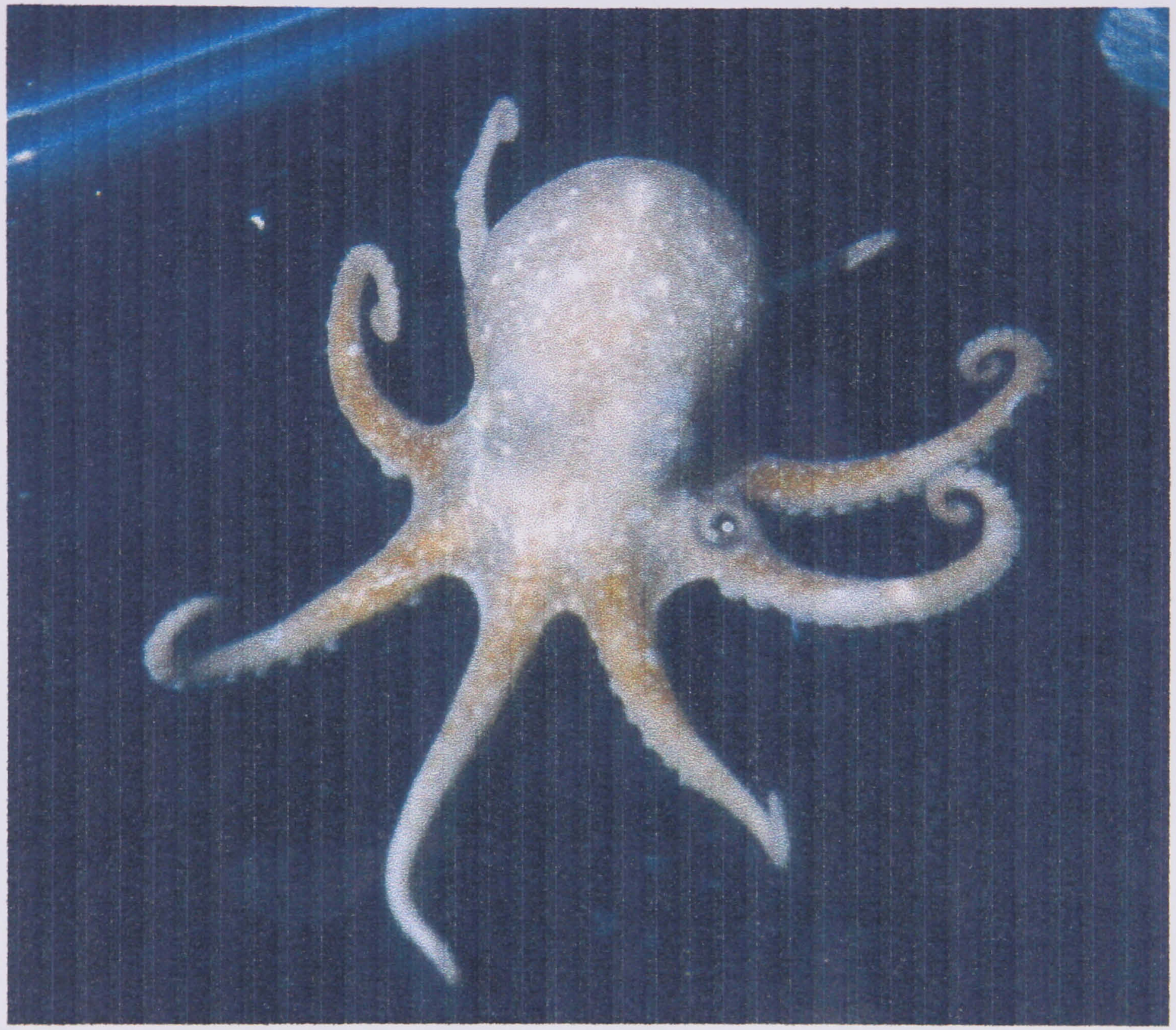


Plate 4.14: *Pareledone* sp. 13 sp. nov.: 47 mm ML.

Pareledone **sp. 14** sp. nov.

Plate 4.15, Figure 4.6

Material examined

Holotype: Male to be selected from material below. Catalogue number BMNH1996190 reserved.

Paratype: Female to be selected from material below. Catalogue number BMNH1996191 reserved.

Other material: **Antarctic Peninsula**, PFS *Polarstern*, 27 November - 4 December 1996. —61°42'S 59°10'W, 807 m, Stn 42/045: AP1962 (M2, 50 mm ML), AP1963 (F2, 57 mm ML). —61°37'S 58°46'W, 583 m, Stn 42/047: AP2032 (F1, 54 mm ML). —61°35'S 58°45'W, 785 m, Stn 42/048: AP2043 (M3, 85 mm ML), AP2044 (F3, 101 mm ML), AP2045 (F2, 72 mm ML), AP2046 (M3, 79 mm ML), AP2047 (F2, 67 mm ML), AP2048 (M3, 73 mm ML), AP2059 (M3, 76 mm ML), AP2060 (M3, 67 mm ML), AP2061 (M2, 68 mm ML), AP2062 (M3 68 mm ML), AP2064 (F1, 62 mm ML), AP2065 (M1, 61 mm ML), AP2066 (F1, 55 mm ML), AP2067 (M1, 61 mm ML), AP2068 (F1, 61 mm ML), AP2079 (M3, 64 mm ML), AP2086 (F1, 54 mm ML). —61°48'S 59°32'W, 555 m, Stn 42/063: AP2142 (M3, 63 mm ML).

Diagnosis. Arms long. Hectocotylised arm with 31-38 suckers. Gills with 9-11 lamellae per demibranch. Skin smooth, loose, wrinkled. Minor papillae in ocular region. No enlarged papillae.

Description. Animals medium sized (ML to 101 mm; TL to 281 mm). Mantle spherical to ovoid (MWI 73.3-86.4-101.9) (Figure 4.6a). Eyes medium sized (EDI 21.8-25.8-31.5). Funnel large (Figure 4.6b) (FuLI 31.6-38.3-46.6) gently tapered (Figure 4.6c). Arms long (MAI 36.9-44.6-50.8). Arm lengths subequal, arm order usually 4=3.2.1 (ALI L1 168.3-208.8-260.0; L2 179.4-212.7-269.1; L3 175.0-217.0-267.3; L4 193.7-219.3-270.9). Suckers small (ASI 4.7-5.8-7.4). Third right arm of males hectocotylised, shorter than opposite number (OAI 79.9-89.5-105.0). Ligula medium sized (LLI 5.0-6.0-7.1). Calamus distinct (Figure 4.6d). Hectocotylised arm with 31-38 suckers, opposite arm with 37-57 suckers. Web of medium depth (WDI 18.4-22.2-28.4), web formula usually B=C=D.A=E. Gills with 9-11 lamellae per demibranch (mode: inner gill lamellae count 10, outer gill lamellae count 10.) Only one mature female captured. Mature ovarian eggs large, 18 mm x 12 mm (EgLI 17.8;

EgWI 11.9) (Figures 4.6h, i). Spermatophores long (SpLI 81.0-98.3-120.6), slender (SpWI 3.8-4.5-5.3) (Figure 4.6f, g, j). Males mature at approximately 65-70 mm ML, females at approximately 100 mm ML.

The integument is smooth, loose and often wrinkled, with occasional minor papillae especially in the ocular region. There are no enlarged papillae present in the ocular region or anywhere else. Densely packed brown / green chromatophores cover the dorsal and ventral surfaces; the demarcation between the two surfaces is faint. The dorsal surface is covered with whitish green markings, with more distinct white markings often present between the eyes. The ventral surface is uniformly brown green with just two white areas around the funnel (Figure 4.6b). Large brown subdermal chromatophores are present on the dorsal and ventral surfaces of the brachial crown.

Distribution. Antarctic Peninsula [61°35'-65°55'S 58°45'-67°40'W] (this study). The recorded depth range of this species is 438-862 m.

Remarks. This species can be distinguished from the other smooth skinned *Pareledone* by its slightly higher gill lamellae count (10 cf. 9), and by its slightly shorter spermatophores, as well as by integumental patterning.

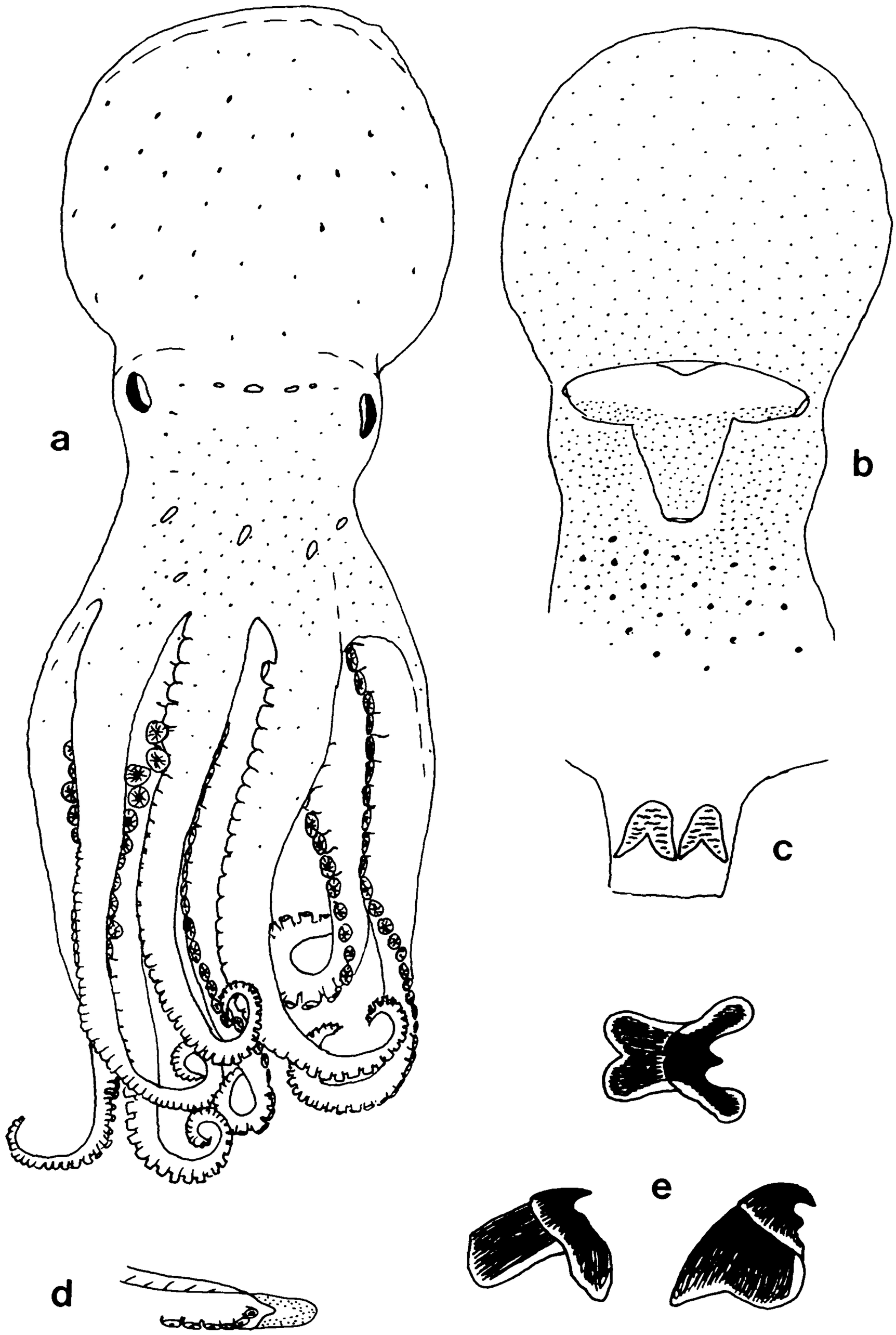


Figure 4.6a-e: *Pareledone* sp. 14 sp. nov.: a, dorsal view of whole animal b, ventral view of mantle and funnel c, funnel organ d, hectocotylus e, beak. All illustrations actual size except beak, twice actual size.

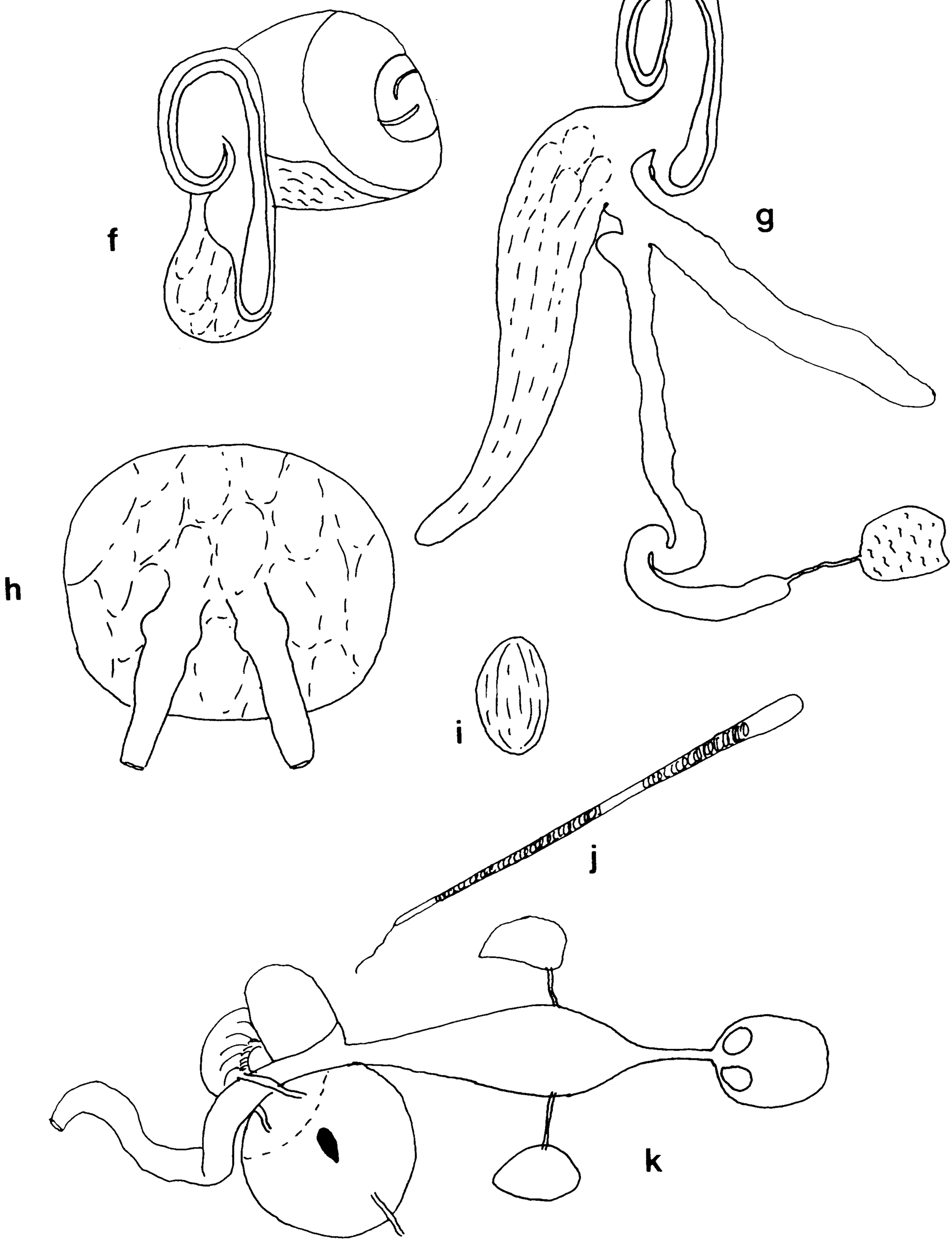


Figure 4.6f-k: *Pareledone* sp. 14 sp. nov.: f, male reproductive system as positioned in situ g, male reproductive organs dissected out h, female reproductive system i. mature ovarian egg j, mature spermatophore k. digestive system. All illustrations actual size.

Plate 4.15: *Pareledone* sp. 14 sp. nov.: 50 mm ML.

Pareledone sp. 15 sp. nov.

Plate 4.16, Figure 4.7

Material examined.

Holotype: Male to be selected from material below. Catalogue number BMNH1996192 reserved.

Paratype: Female to be selected from material below. Catalogue number BMNH1996193 reserved.

Other material: **Antarctic Peninsula**, PFS *Polarstern*, 23-26 November 1996. —60°50'S 55°42'W, 302 m, Stn 42/029: AP983 (M3, 46 mm ML), AP984 (F3, 46 mm ML), AP1074 (M3, 39 mm ML), AP1075 (M3, 41 mm ML), AP1076 (M3, 42 mm ML), AP1077 (M3, 43 mm ML), AP1078 (M3, 43 mm ML). —60°51'S 55°33'W, 280 m, Stn 42/033: AP1241 (F2, 38 mm ML). —60°58'S 55°12'W, 323 m, Stn 42/036: AP1444 (M3, 42 mm ML), AP1445 (M3, 42 mm ML), AP1446 (M3, 40 mm ML). —61°01'S 54°49'W, 503 m, Stn 42/040: AP1767 (F3, 55 mm ML), AP1770 (F3, 35 mm ML), AP1771 (M3, 46 mm ML), AP1772 (M3, 43 mm ML), AP1773 (F3, 54 mm ML), AP1774 (F3, 54 mm ML), AP1775 (M3, 42 mm ML), AP1777 (M3, 50 mm ML), AP1779 (M3, 42 mm ML), AP1780 (M3, 41 mm ML), AP1783 (M3, 38 mm ML). —61°04'S 54°39'W, 374 m, Stn 42/041: AP1826 (M3, 32 mm ML).

Diagnosis. Arms medium to long. Hectocotylised arm with 25-33 suckers. Gills with 6-8 lamellae per demibranch. Large closely set papillae on dorsal surface. Enlarged white supraocular papillae.

Description. Animals small to medium sized (ML to 54 mm; TL to 151 mm). Mantle spherical to ovoid (MWI 76.0-85.8-104.8) (Figure 4.7a). Eyes medium (EDI 20.4-24.7-31.6). Funnel large (Figure 4.7b) (FuLI 32.6-45.3-57.1) gently tapered (Figure 4.7c). Arms medium to long (MAI 41.6-49.2-57.5). Arm lengths subequal, arm order usually 2.3.4.1 (ALI L1 131.5-186.8-233.3; L2 168.0-202.0-270.0; L3 159.4-196.1-238.5; L4 137.5-190.7-238.5). Suckers small (ASI 4.3-5.9-7.5). Third right arm of males hectocotylised, shorter than opposite number (OAI 78.1-87.6-113.7). Ligula medium sized (LLI 7.0-8.9-14.0). Calamus distinct (Figure 4.7d). Hectocotylised arm with 25-33 suckers, opposite arm with 37-50 suckers. Web of medium depth (WDI 18.3-23.9-28.9), web formula usually C.B=D.A=E. Gills with 6-8 lamellae per demibranch (mode: inner gill lamellae count 7, outer gill lamellae

count 7.) Mature ovarian eggs large, 16 mm x 7 mm (EgLI 21.7-25.7-29.6; EgWI 8.7-10.5-13.0) (Figures 4.7h, i). Spermatophores long (SpLI 107.1-125.9-150.0), slender (SpWI 3.4-5.4-6.4) (Figures 4.7f, g, j). Males mature at approximately 35-40 mm ML, females at approximately 50-55 mm ML.

The integumental sculpture consists of large, closely set papillae over the entire dorsal surface. The papillae are highly distinctive in shape; each large papilla appears to consist of a circular arrangement of very small papillae, hence a large papilla is 'taller' at the edges than at the centre (Figure 4.7b, Plate 4.16). The ventrolateral ridge is highly conspicuous and papillae and chromatophores extend only a few millimetres beyond it. The chromatophores give a pink brown colouration to the dorsal surface; the ventral surface is creamy white. Enlarged supraocular papillae are present and are highly conspicuous because of their white colouration.

Distribution. Antarctic Peninsula [60°50'-61°19'S 54°38'-56°34'W] (this study). The recorded depth range of this species is 268-512 m.

Remarks. The structure of the papillae is sufficient to distinguish this species from all other described species of *Pareledone*.

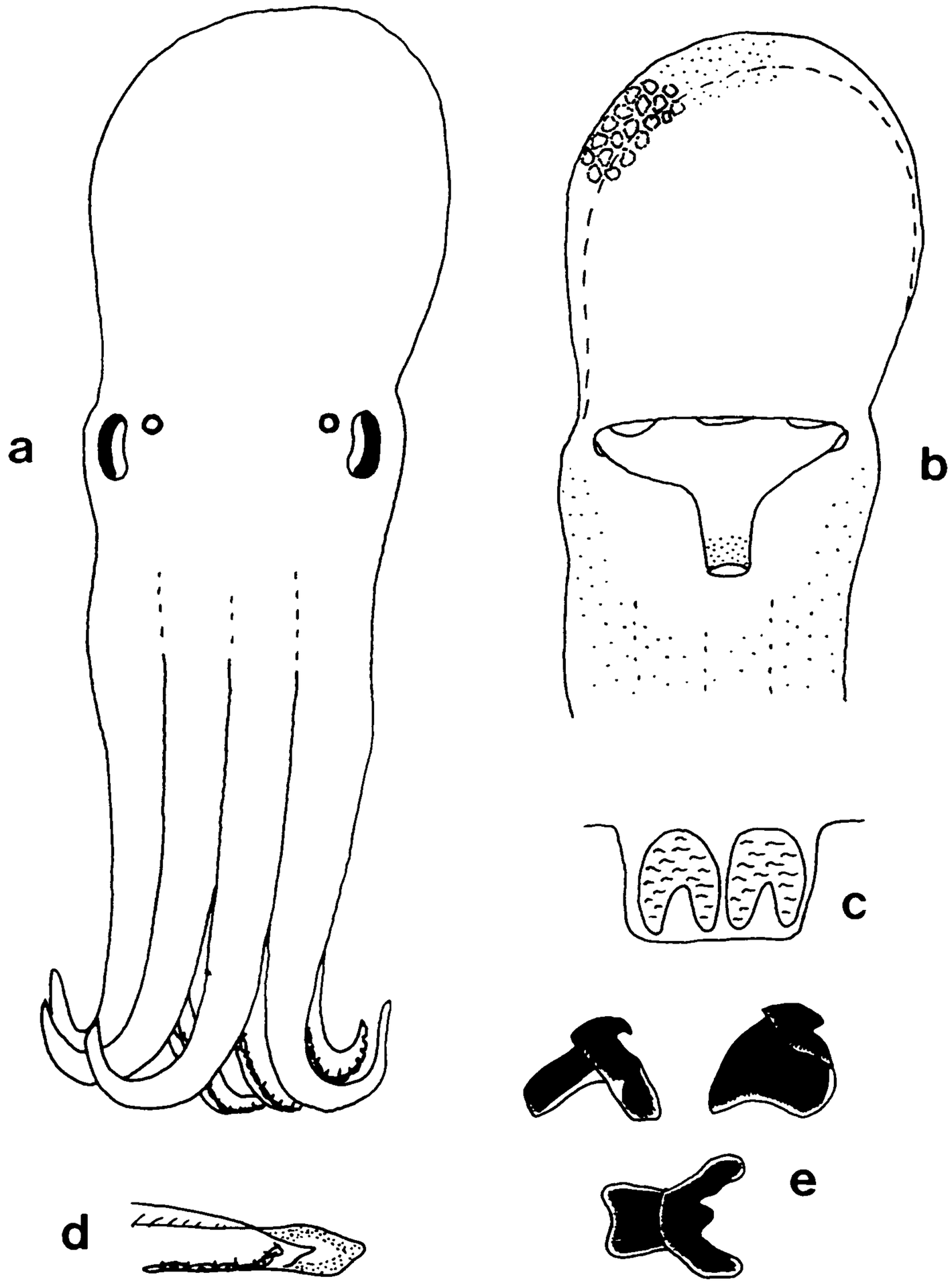


Figure 4.7a-e: *Pareledone* sp. 15 sp. nov.: a, dorsal view of whole animal b, ventral view of mantle and funnel c, funnel organ d, hectocotylus e, beak. All illustrations actual size except beak, twice actual size.

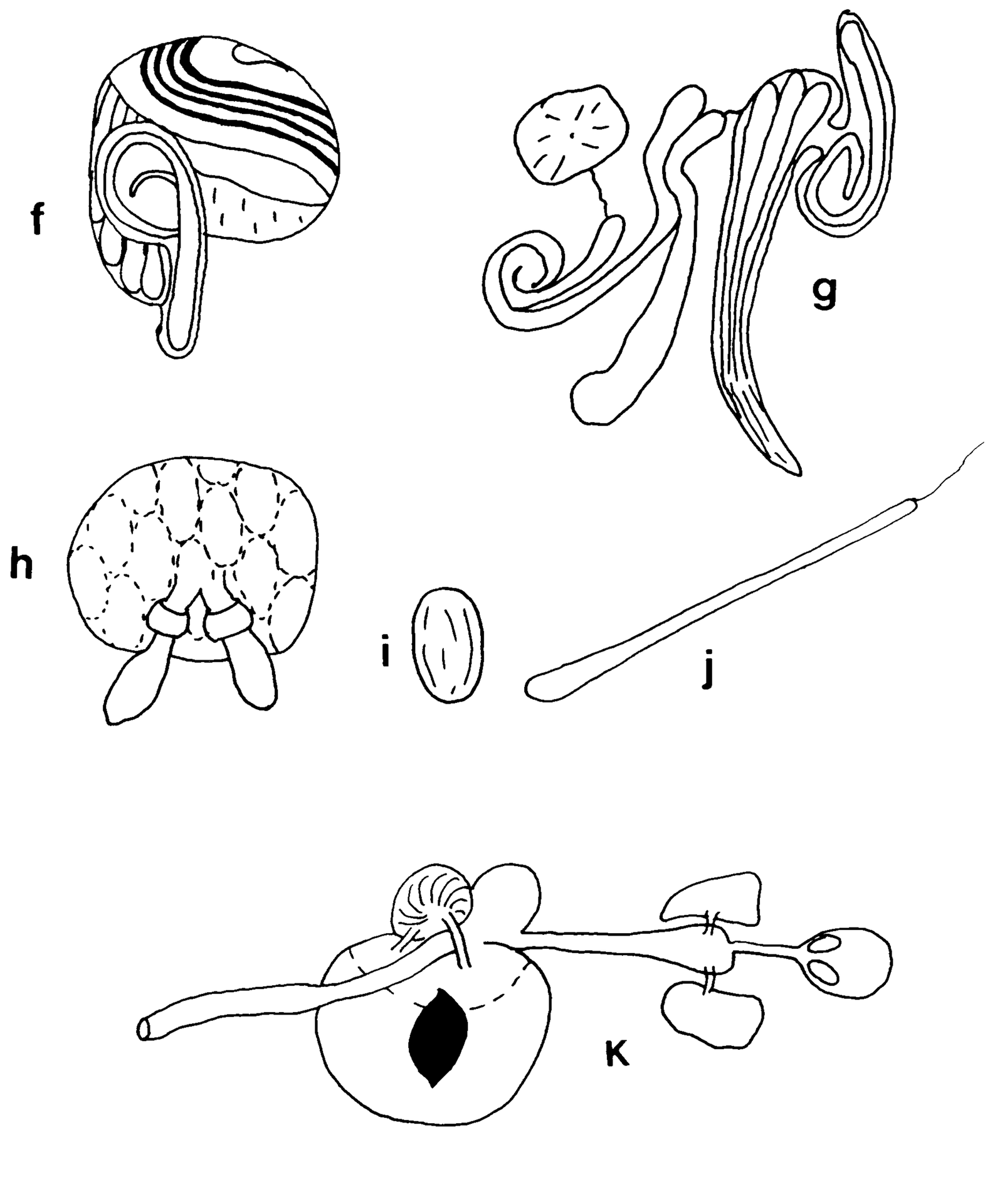


Figure 4.7f-k: *Pareledone* sp. 15 sp. nov.: f, male reproductive system as positioned in situ g, male reproductive organs dissected out h, female reproductive system i, mature ovarian egg j, mature spermatophore k, digestive system. All illustrations actual size.

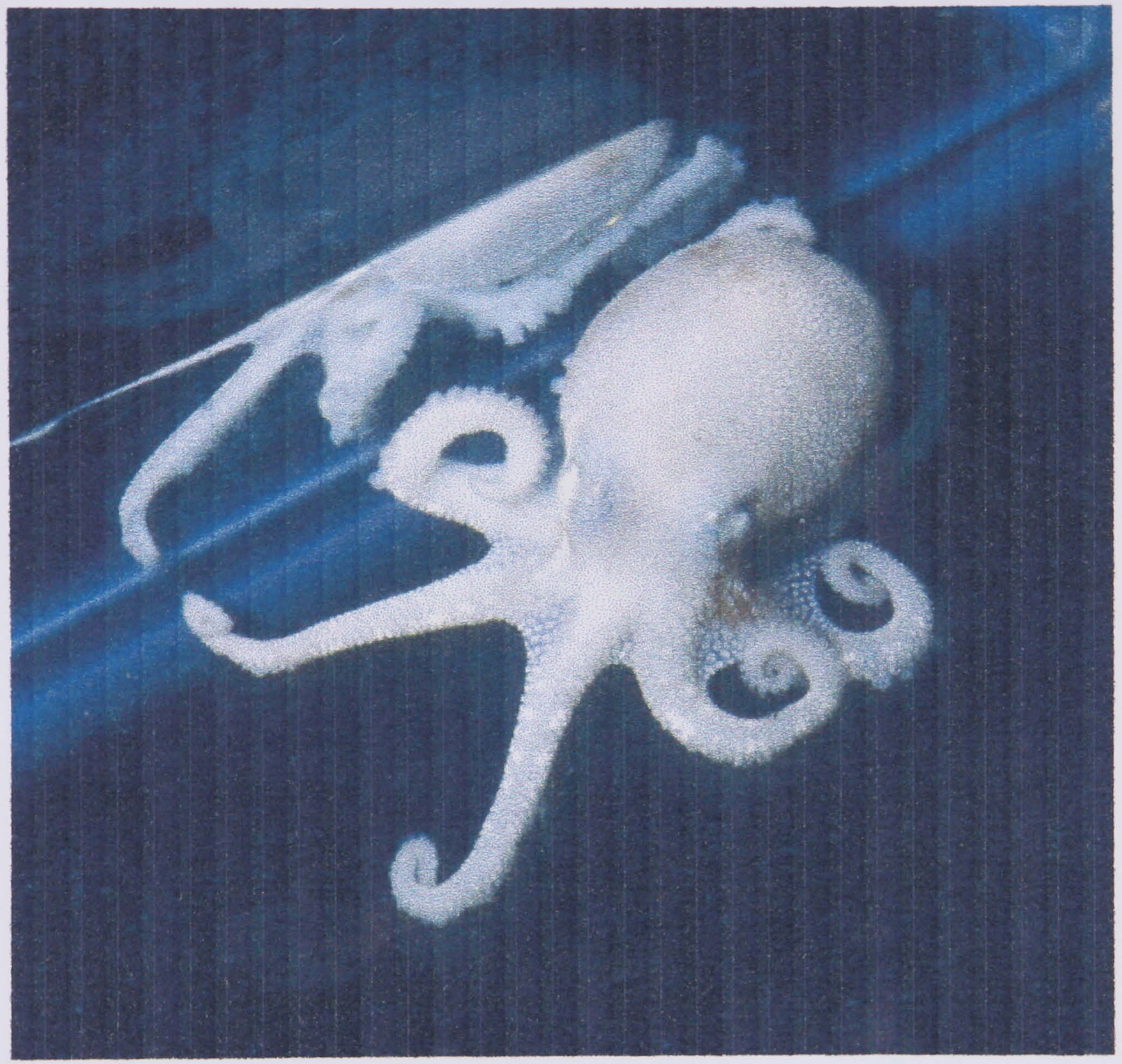


Plate 4.16 *Pareledone* sp. 15 sp. nov.: 46 mm ML.

Pareledone **sp. 16** sp. nov.

Plate 4.17, Figure 4.8

Material examined.

Holotype: Male to be selected from material below. Catalogue number BMNH1996194 reserved.

Paratype: Female to be selected from material below. Catalogue number BMNH1996195 reserved.

Other material: **Antarctic Peninsula**, PFS *Polarstern*, 16 November - 9 December 1996. —61°08'S 56°11'W, 380 m, Stn 42/003: AP3 (M3, 43 mm ML). —61°11'S 55°58'W, 130 m, Stn 42/005: AP116 (M3, 46 mm ML), AP117 (M3, 45 mm ML). —61°15'S 55°52'W, 155 m, Stn 42/008: AP136 (F3, 63 mm ML), AP137 (M3, 47 mm ML), AP138 (M3, 55 mm ML), AP139 (M3, 48 mm ML), AP140 (M3, 54 mm ML). —61°14'S 55°41'W, 95 m, Stn 42/010: AP175 (F3, 64 mm ML). —61°13'S 55°45'W, 98 m, Stn 42/011: AP291 (M3, 48 mm ML), AP292 (M3, 45 mm ML), AP293 (M3, 46 mm ML), AP294 (M3, 43 mm ML), AP295 (M3, 47 mm ML), AP296 (M3, 43 mm ML), AP297 (F3, 45 mm ML). —61°06'S 55°59'W, 213 m, Stn 42/023: AP740 (F3, 70 mm ML). —61°05'S 55°56'W, 174 m, Stn 42/024: AP773 (M3, 42 mm ML), AP774 (M3, 54 mm ML), AP775 (M3, 56 mm ML), AP776 (M3, 45 mm ML), AP777 (M3, 48 mm ML), AP778 (M3, 41 mm ML), AP779 (M3, 48 mm ML). —61°07'S 55°50'W, 100 m, Stn 42/025: AP834 (F3, 56 mm ML), AP835 (F3, 65 mm ML), AP836 (F3, 58 mm ML), AP837 (F3, 58 mm ML), AP838 (F3, 67 mm ML), AP839 (F3, 60 mm ML), AP840 (F3, 66 mm ML), AP841 (F3, 59 mm ML), AP842 (F3, 59 mm ML). —61°02'S 55°52'W, 148 m, Stn 42/026: AP858 (F3, 61 mm ML). —60°57'S 55°33'W, 80 m, Stn 42/078: AP2194 (F3, 55 mm ML), AP2195 (F3, 55 mm ML), AP2196 (F3, 62 mm ML), AP2197 (F3, 59 mm ML), AP2198 (F3, 67 mm ML). —60°54'S 55°37'W, 120 m, Stn 42/079: AP2229 (F3, 56 mm ML).

Diagnosis. Arms short. Hectocotylised arm with 23-29 suckers. Gills with 6-9 lamellae per demibranch. Patch and groove type markings on dorsal surface, with pale brown oblong marking extending from brachial crown to mid dorsum.

Description. Animals medium sized (ML to 67 mm; TL to 176 mm). Mantle spherical to ovoid (MWI 73.2-88.6-100.0) (Figure 4.8a). Eyes medium sized (EDI 14.8-20.5-27.1). Funnel large (Figure 4.8b) (FuLI 33.9-42.0-65.3) gently tapered (Figure 4.8c). Arms short (MAI 47.4-59.9-74.2). Arm lengths subequal, arm order usually 3.4.2.1 (ALI L1 119.4-144.3-171.1; L2 121.2-157.6-186.7; L3 125.0-165.3-211.1; L4 129.7-161.3-191.1). Suckers medium sized (ASI 5.5-7.3-9.5). Third right arm of males hectocotylised, shorter than opposite number (OAI 77.0-86.4-94.9).

Ligula medium sized (LLI 8.0-11.9-14.9). Calamus distinct (Figure 4.8d). Hectocotylised arm with 23-29 suckers; opposite arm with 35-41 suckers. Web slightly deeper (WDI 28.8-35.4-41.2), web formula usually C=D.B.A=E. Gills with 6-9 lamellae per demibranch (mode: inner gill lamellae count 8, outer gill lamellae count 8.) Mature ovarian eggs large, 20 mm x 8 mm (EgLI 20.0-27.0-32.1; EgWI 6.2-9.7-12.7) (Figures 4.8h, i). Spermatophores long (SpLI 97.7-119.4-150.0), slender (SpWI 4.3-5.3-6.4) (Figures 4.8f, g, j). Males mature at approximately 45-50 mm ML, females at approximately 55-60 mm ML.

The integumental sculpture consists of closely set papillae on the dorsal surface. These papillae consist of a raised area approximately 1.5 mm in diameter with a secondary nipple-like projection in the centre. The nipple is a similar colour to the area between the papillae, whilst the remainder of the papilla is lighter in colour (Figure 4.8b, Plate 4.17). Densely packed chromatophores on the dorsal surface give rise to a brown colouration. A paler brown, large oblong marking is usually present, extending from the brachial crown, up between the eyes to the middle of the dorsal mantle. On stimulation this species may turn to a deep red colour. The ventrolateral integumentary ridge is well defined. Chromatophores and papillae cease at this ridge. The ventral surface is smooth and white (rather than the creamy colour of mantle muscle seen on the ventral surface of most species). Small white markings may be apparent on the arms.

Distribution. Antarctic Peninsula [60°50'-61°21'S 54°44'-56°11'W] (this study). The recorded depth range of this species is 70-419 m.

Remarks. The structure of the papillae is sufficient to distinguish this species from all other described species of *Pareledone.*

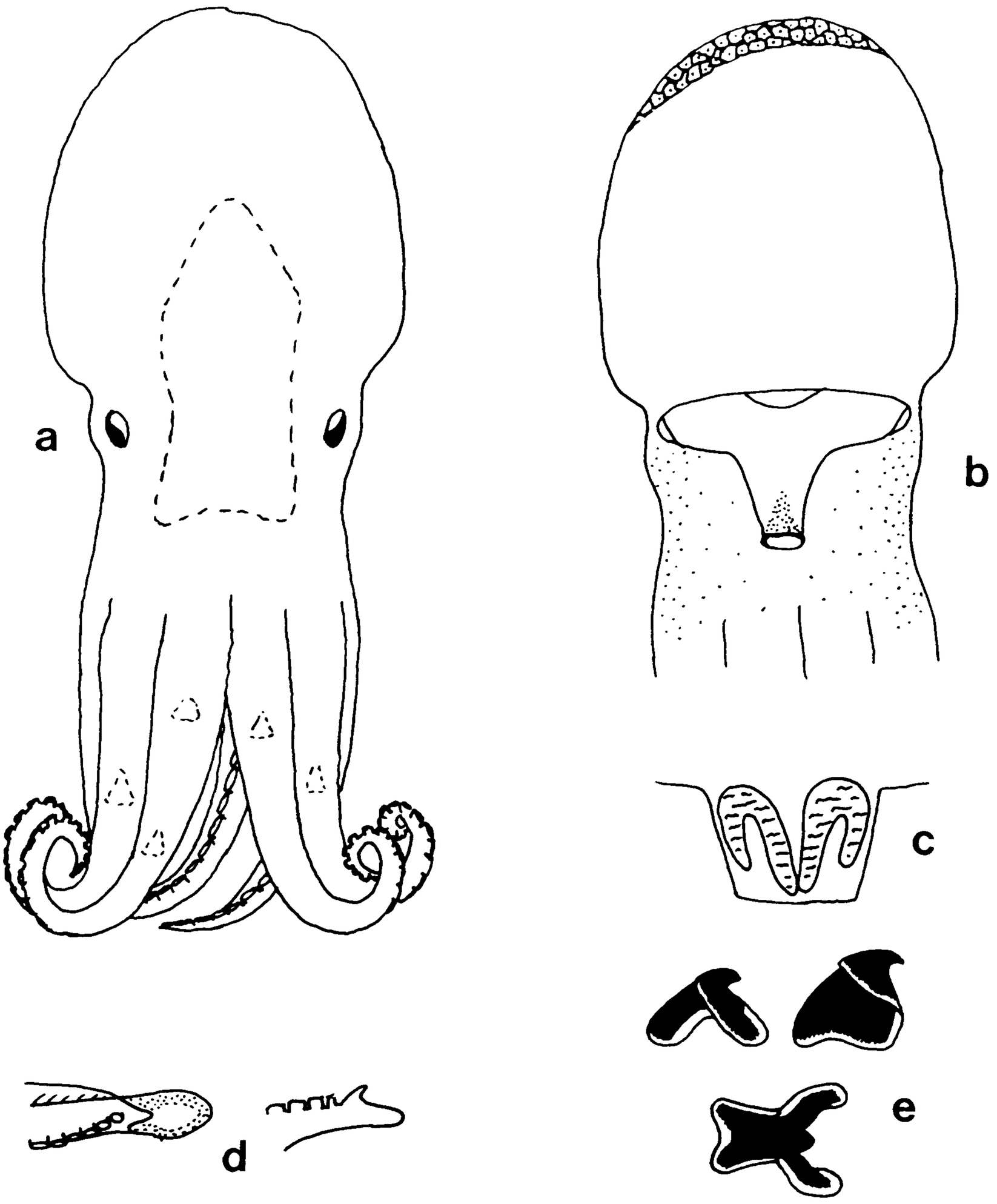


Figure 4.8a-e: *Pareledone* sp. 16 sp. nov.: a, dorsal view of whole animal b, ventral view of mantle and funnel c, funnel organ d, hectocotylus e, beak. All illustrations actual size except beak, twice actual size.

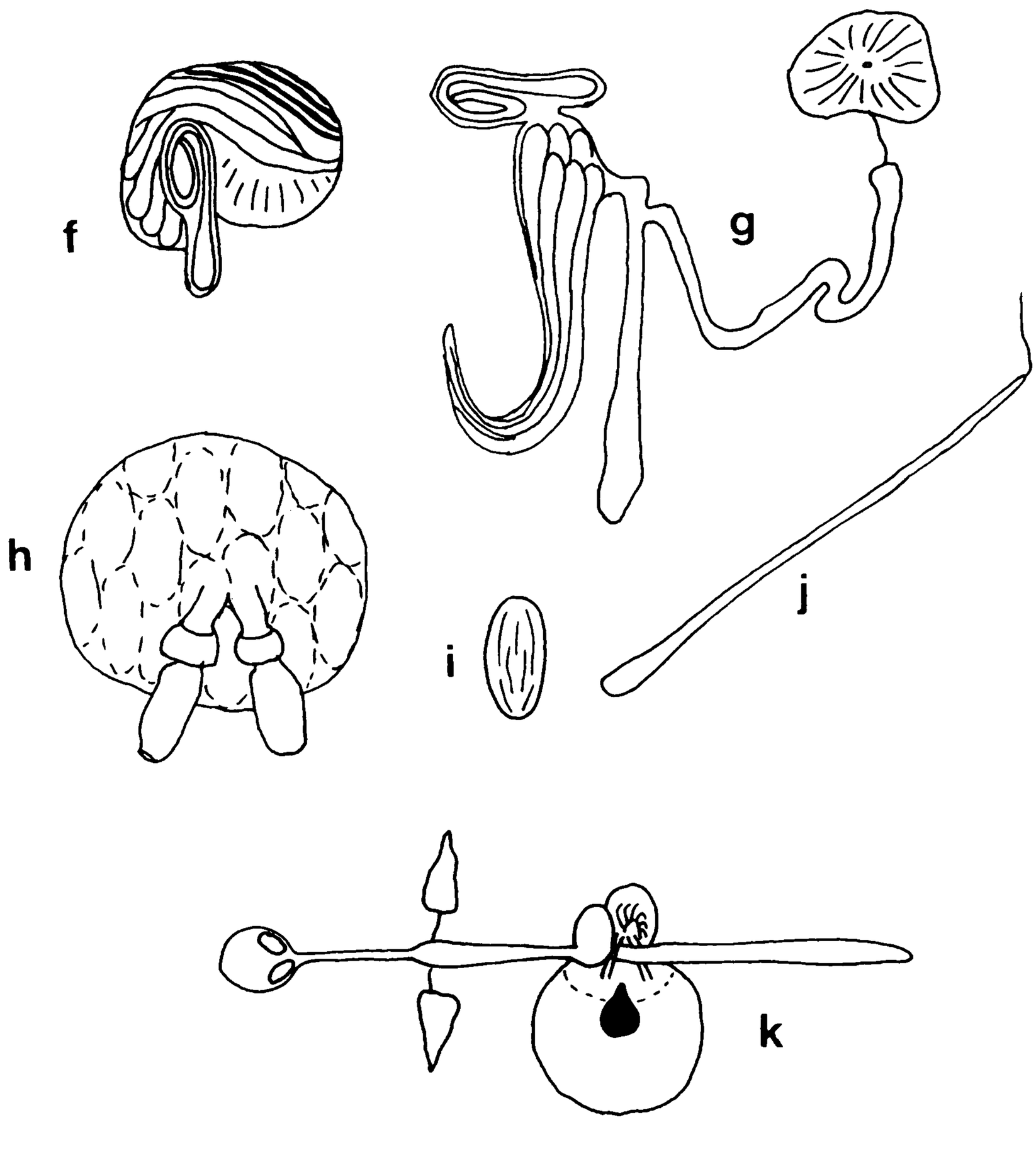


Figure 4.8f-k: *Pareledone* sp. 16 sp. nov.: f, male reproductive system as positioned in situ g, male reproductive organs dissected out h, female reproductive system i, mature ovarian egg j, mature spermatophore k, digestive system. All illustrations actual size.

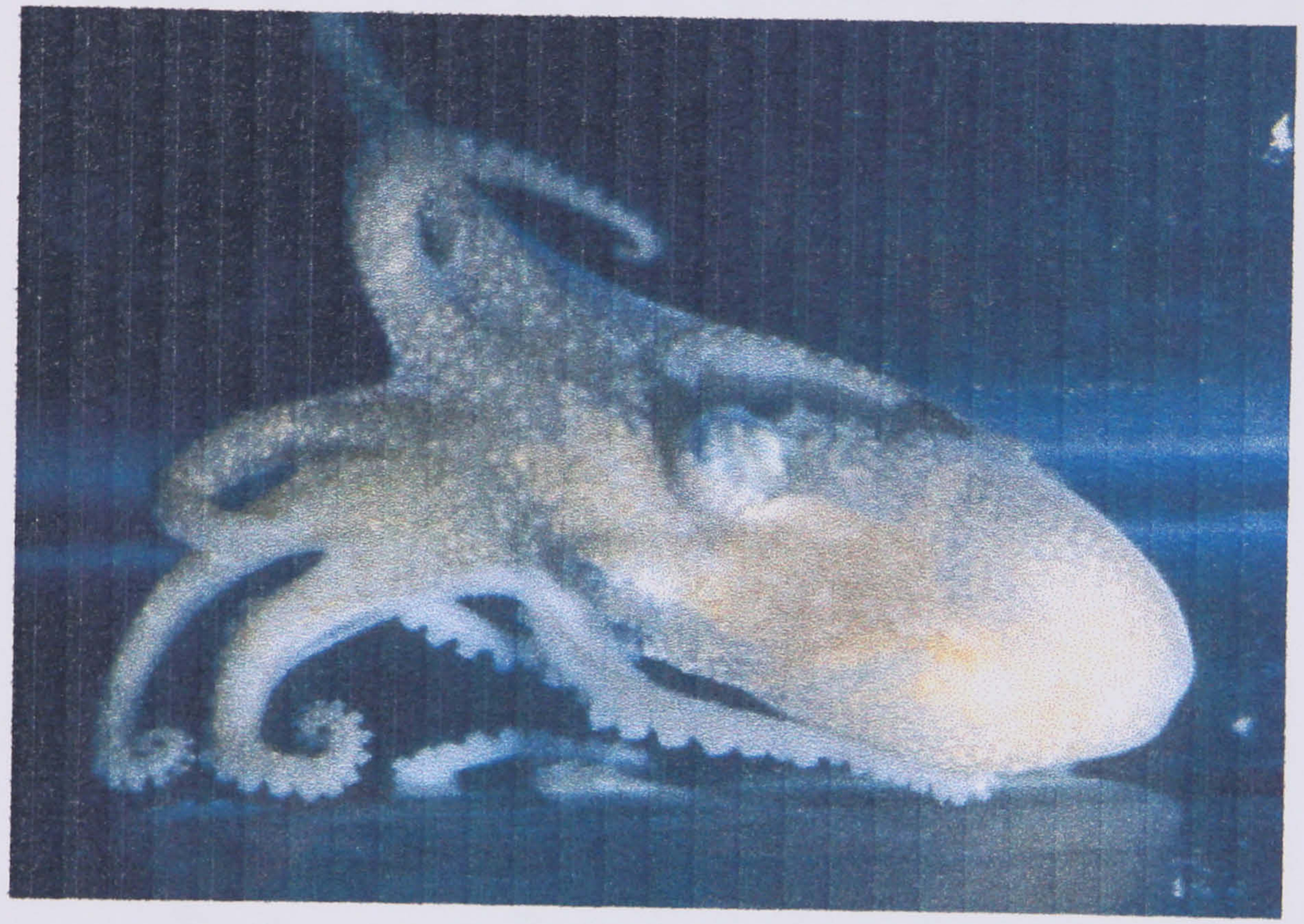


Plate 4.17 *Pareledone* sp. 16 sp. nov.: 44 mm ML.

Pareledone **sp. 19** sp. nov.

Plate 4.18, Figure 4.9

Material examined.

Holotype: Male to be selected from material below. Catalogue number BMNH1996196 reserved.

Paratype: Female to be selected from material below. Catalogue number BMNH1996197 reserved.

Other material: **Antarctic Peninsula**, PFS *Polarstern*, 17-24 November 1996. —61°11'S 55°58'W, 130 m, Stn 42/005: AP125 (F3, 64 mm ML). —61°15'S 55°52'W, 155 m, Stn 42/008: AP142 (M3, 59 mm ML), AP143 (M3, 44 mm ML), AP144 (M3, 43 mm ML), AP146 (M3, 45 mm ML). —61°16'S 55°56'W, 173 m, Stn 42/009: AP163 (M3, 55 mm ML). —61°17'S 54°53'W, 200 m, Stn 42/012: AP333 (M3, 46 mm ML), AP335 (M3, 42 mm ML), AP336 (M3, 44 mm ML), AP338 (M3, 45 mm ML), AP340 (M3, 41 mm ML). —61°21'S 54°44'W, 291 m, Stn 42/013: AP351 (M3, 37 mm ML), AP352 (M3, 38 mm ML), AP353 (M3, 38 mm ML), AP354 (M3, 38 mm ML). —61°06'S 55°59'W, 213 m, Stn 42/023: AP662 (M3, 47 mm ML), AP663 (M3, 51 mm ML), AP664 M3, 55 mm ML), AP665 (M3, 46 mm ML), AP689 (F3, 56 mm ML). —61°02'S 55°52'W, 148 m, Stn 42/026: AP853 (F3, 62 mm ML). —60°50'S 55°42'W, 302 m, Stn 42/029: AP1062 (F3, 63 mm ML), AP1064 (F3, 63 mm ML), AP1066 (F3, 60 mm ML), AP1067 (F3, 55 mm ML), AP1068 (F3, 58 mm ML), AP1069 (F3, 60 mm ML). —60°54'S 55°46'W, 219 m, Stn 42/030: AP1097 (F3, 48 mm ML), AP1098 (F3, 59 mm ML). —60°55'S 55°46'W, 235 m, Stn 42/031: AP1137 (F3, 62 mm ML). —60°51'S 55°33'W, 280 m, Stn 42/033: AP1223 (F3, 66 mm ML), AP1224 (F3, 72 mm ML), AP1225 (F3, 58 mm ML). —60°52'S 55°29'W, 283 m, Stn 42/034: AP1294 (F3, 63 mm ML), AP1295 (F3, 56 mm ML), AP1297 (F3, 60 mm ML), AP1298 (F3, 66 mm ML), AP1299 (F3, 60 mm ML).

Diagnosis. Arms medium length. Hectocotylised arm with 27-33 suckers. Gills with 7-9 lamellae per demibranch. Large closely set papillae on dorsal surface. Two pairs of very large supraocular papillae.

Description. Animals medium sized (ML to 72 mm; TL to 203 mm). Mantle spherical to ovoid (MWI 75.8-89.1-102.2) (Figure 4.9a). Eyes medium sized (EDI 16.7-24.3-35.1). Funnel large (Figure 4.9b) (FuLI 32.3-42.3-50.0) gently tapered (Figure 4.9c). Arms medium length (MAI 46.3-55.9-66.7). Arm lengths subequal, arm order usually 4=3.2.1 (ALI L1 116.1-159.7-210.5; L2 138.7-168.2-205.3; L3 144.6-175.2-210.5; L4 143.5-177.0-208.9). Suckers small (ASI 4.3-6.0-8.1). Third right arm of males hectocotylised, shorter than opposite number (OAI 75.0-86.1-

93.7). Ligula medium sized (LLI 6.8-8.7-10.3). Calamus distinct (Figure 4.9d). Hectocotylised arm with 27-33 suckers, opposite arm with 40-54 suckers. Web of medium depth (WDI 35.5-29.8-34.8), web formula usually C=D.B.A=E. Gills with 7-9 lamellae per demibranch (mode: inner gill lamellae count 8, outer gill lamellae count 7.) Mature ovarian eggs large, 17 mm x 9 mm (EgLI 17.9-24.2-29.2; EgWI 5.4-11.2-15.0) (Figures 4.9h, i). Spermatophores long (SpLI 123.7-150.0-188.9), slender (SpWI 4.2-5.2-5.9) (Figures 4.9f, g, j). Males mature at approximately 40-45 mm ML, females at approximately 55-60 mm ML.

The integumental sculpture consists of large, rounded, closely set papillae over the entire dorsal surface. There are two very large supraocular papillae associated with each eye, the posterior being slightly larger (Plate 4.18). This papilla may be as large as 8 mm in height but the animal has the ability to retract these enlarged papillae so that they are barely distinguishable. Other enlarged papillae (usually between three and seven) are present on the dorsal mantle. There is apparently no characteristic arrangement of these but this may be disguised by the retraction of papillae. Papillae cease at, or soon after, the inconspicuous ventrolateral integumentary ridge. Pale brown chromatophores on the dorsal surface continue only a few millimetres over the ventrolateral ridge; the ventral surface is creamy white. White markings consist of a highly irregular shaped bar between the eyes and other apparently random splashes of white on the dorsal mantle. Papillae are more sparsely distributed in the white areas.

Distribution. Antarctic Peninsula [60°50'-61°47'S 54°38'-57°30'W] (this study). The recorded depth range of this species is 121-365 m.

Remarks. This species may be distinguished from other papillated species by the two enlarged supraocular papillae. Although two other species have a single enlarged supraocular papilla, *P. framensis* also differs by having papillae on the ventral surface, and *P.* sp. 10 also differs in the arrangement of white markings.

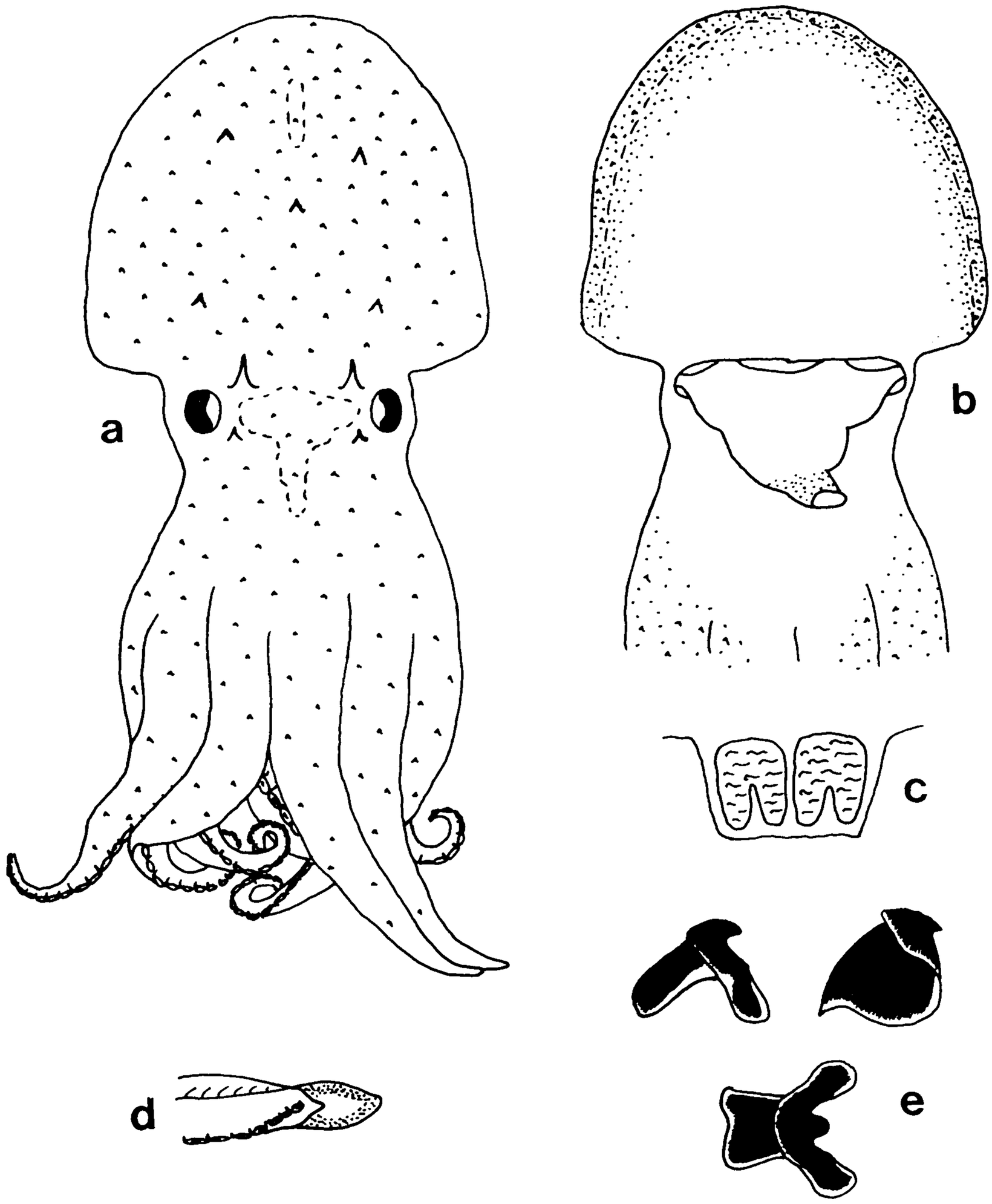
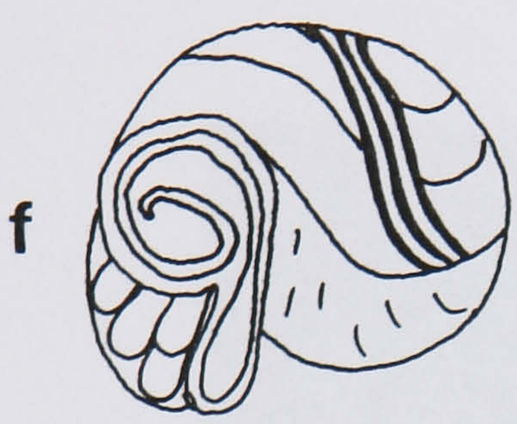
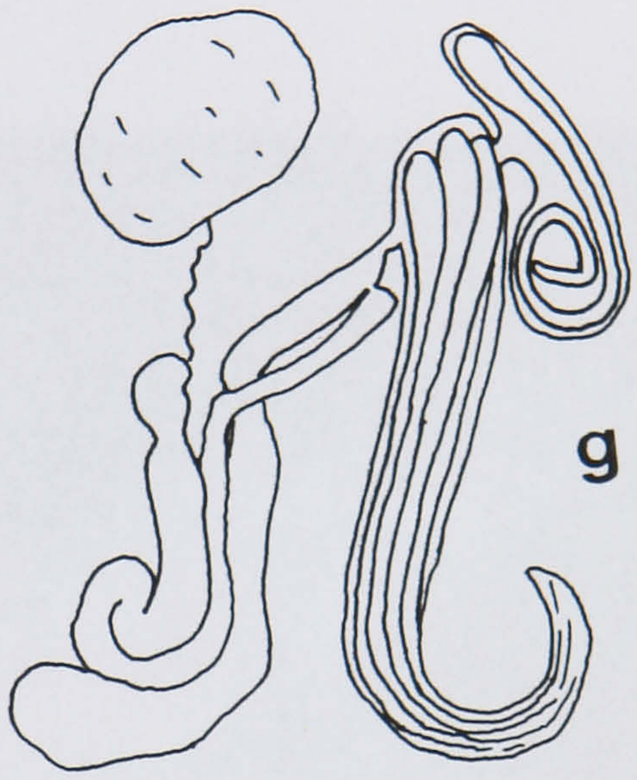
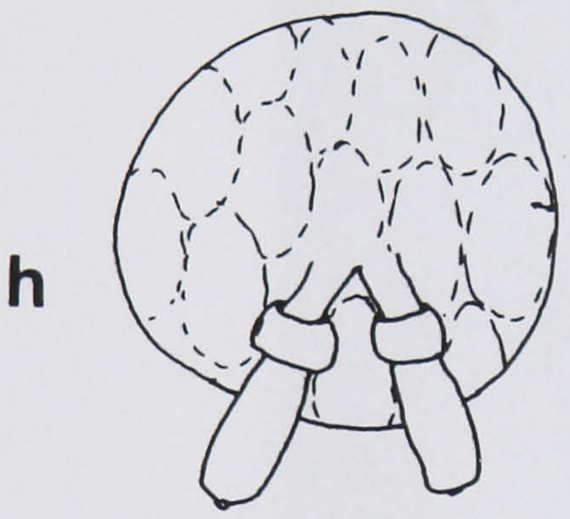


Figure 4.9a-e: *Pareledone* sp. 19 sp. nov.: a, dorsal view of whole animal b, ventral view of mantle and funnel c, funnel organ d, hectocotylus e, beak. All illustrations actual size except beak, twice actual size.







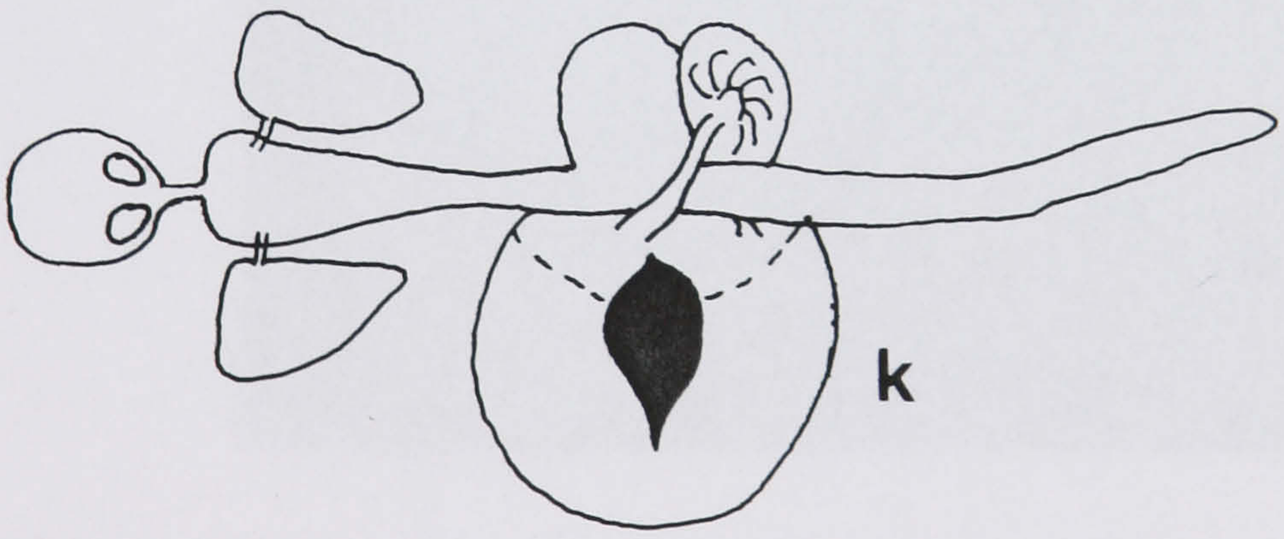


Figure 4.9f-k: *Pareledone* sp.19 sp. nov.: f, male reproductive system as positioned in situ g, male reproductive organs dissected out h, female reproductive system i, mature ovarian egg j, mature spermatophore k, digestive system. All illustrations actual size.

Plate 4.18 *Pareledone* sp. 19 sp. nov.: 65 mm ML.

"Pareledone" polymorpha (Robson)

Plate 4.19, Figure 4.10

Graneledone polymorpha, Robson, 1930: 390, pl 3 fig 1.

Pareledone polymorpha, Robson, 1932: 276, pl 5 fig 1. –Roper *et al.*, 1985: 202, 3 figs. –Dong, 1991: 184, text fig 2.

Material examined. **Weddell Sea**, PFS *Polarstern*, 5-16 February 1996. —71°03'S 11°25'W, 462 m, Stn 39/001: WS2 (M2, 59 mm ML), WS3 (M2, 54 mm ML), WS4 (M1, 39 mm ML), WS5 (M1, 43 mm ML), WS43 (F1, 35 mm ML), WS52 (M1, 54 mm ML), WS53 (F1, 40 mm ML), WS57 (F1, 31 mm ML), WS58 (M1, 38 mm ML), WS60 (F1, 41 mm ML), WS69 (M1, 43 mm ML), WS70 (M1, 48 mm ML), WS98 (M1, 46 mm ML), WS99 (M1, 57 mm ML). —71°41'S 12°44'W, 227 m, Stn 39/005: WS113 (F1, 45 mm ML). —73°23'S 21°11'W, 338 m, Stn 39/011: WS206 (F1, 36 mm ML), WS207 (M1, 37 mm ML), WS208 (M1, 35 mm ML), WS209 (F1, 33 mm ML). —73°42'S 22°31'W, 446 m, Stn 39/015: WS279 (F2, 69 mm ML). —73°18'S 21°10'W, 468 m, Stn 39/017: WS333 (M2, 80 mm ML).

Antarctic Peninsula, PFS *Polarstern*, 19-28 November 1996. —61°14'S 54°38'W, 333 m, Stn 42/014: AP411 (F3, 70 mm ML), AP412 (M2, 62 mm ML). —61°18'S 55°59'W, 296 m, Stn 42/017: AP481 (M2, 64 mm ML). —61°30'S 56°15'W, 572 m, Stn 42/019: AP530 (F3, 62 mm ML). —61°23'S 56°07'W, 316 m, Stn 42/020: AP553 (M2, 48 mm ML), AP554 (M2, 52 mm ML). —60°50'S 55°42'W, 302 m, Stn 42/029: AP1053 (M3, 82 mm ML). —60°52'S 55°29'W, 283 m, Stn 42/034: AP1357 (M2, 78 mm ML), AP1358 (F3, 90 mm ML). —61°01'S 54°49'W, 503 m, Stn 42/040: AP1814 (F3, 88 mm ML), AP1816 (F3, 90 mm ML), AP1817 (M3, 84 mm ML). —61°43'S 59°12'W, 589 m, Stn 42/044: AP1918 (F3, 66 mm ML). —61°37'S 58°46'W, 583 m, Stn 42/047: AP2026 (F3, 90 mm ML), AP 2027 (M3, 84 mm ML). —61°35'S 58°45'W, 785 m, Stn 42/048: AP2069 (M2, 68 mm ML).

Diagnosis. Beak small; rostral tip of lower beak sharp. Ligula groove long, well-marked and deep with transverse ridges. Arms short. Hectocotylised arm with 25-30 suckers. Gills with 6-8 lamellae per demibranch. Widely scattered papillae on dorsal surface. Ventral surface smooth and creamy white. No supraocular papillae.

Description. Animals medium sized (ML to 90 mm; TL to 247 mm). Mantle spherical to ovoid (MWI 64.4-84.5-105.6) (Figure 4.10a). Eyes medium sized (EDI 18.9-26.5-35.9). Funnel large (Figure 4.10b) (FuLI 27.8-35.6-48.2) gently tapered;

funnel organ W-shaped (Figure 4.10c). Arms short (MAI 40.7-59.5-83.8). Arm lengths subequal, arm order usually 3=4.2.1 (ALI L1 95.1-146.3-211.4; L2 106.5-159.5-202.9; L3 116.1-165.2-242.9; L4 116.1-163.5-202.9). Suckers uniserial, small (ASI 4.2-5.7-8.5), without sucker enlargement. Third right arm of males hectocotylised, shorter than opposite number (OAI 81.2-90.4-108.3). Ligula large (LLI 14.3); ligula groove long, well marked and deep, with transverse ridges. Calamus distinct (Figure 4.10d). Hectocotylised arm with 25-30 suckers, opposite arm with 35-50 suckers. Web of medium depth (WDI 17.4-26.5-35.1), web formula usually C.B=D.A=E. Ink sac present. Chromatophores on dorsal surface of digestive gland. Posterior salivary glands large (Figure 4.10k). Gills with 6-8 lamellae per demibranch (mode: inner gill lamellae count 7, outer gill lamellae count 7.) Beak small; rostral tip of lower beak sharp (Figure 4.10e). Mature ovarian eggs large, 15 mm x 7 mm (EgLI 11.4-15.0-22.7; EgWI 5.7-7.4-10.6) (Figures 4.10h, i). Only one undamaged fully mature male examined. Spermatophores shorter (SpLI 70.7), slender (SpWI 3.4) (Figures 4.10f, g, j). Males mature at approximately 75-85 mm ML, females at approximately 70-80 mm ML.

The integument is often loose and gelatinous. The integumental sculpture consists of fine, widely scattered papillae on the dorsal surface. Two short, longitudinal integumentary ridges are present on the mid-dorsal posterior mantle. A ventrolateral integumentary ridge is also present. The ventral surface is smooth and creamy white in colouration with a few scattered chromatophores laterally. Chromatophores are densely packed on the dorsal surface which varies (even on a single animal) from brown to green to blue (Plate 4.19). White markings consist of an inverted V on the dorsal brachial crown and various apparently randomly placed smaller white spots on the dorsal mantle and arms.

Taxonomic Summary

Type Specimen. *Graneledone polymorpha* Robson, 1930. Mature male, 52 mm ML. In Natural History Museum, London. Catalogue number BMNH 1951.4.26.26.

Type Locality. Antarctica. South Georgia. Mouth of Cumberland Bay. RV *Discovery*. 1 April, 1926.

Etymology. From the Greek *polu* (much) and *morphe* (form), because of the variability of mantle shape seen in this species.

Distribution. South Georgia (Cumberland Bay, Wilson Harbour, SE of Jason Light, off Cape Saunders) (Robson, 1930); South Georgia [53°37'-55°26'S 39°33'-34°44'W], Antarctic Peninsula [60°50'-61°51'S 54°38'-59°13'W], Weddell Sea [71°03'-73°42'S 11°26'-22°31'W] (this study). The recorded depth range of this species is 15-862 m.

Remarks. This species may be distinguished form the other *Pareledone* species discussed by its W-shaped funnel organ, by the chromatophores on the dorsal surface of the digestive gland, by the small sharp beak, by the large posterior salivary glands, by the considerably shorter spermatophores, by the large ligula with its transverse ridges and by the two short longitudinal integumentary ridges present on the mid-dorsal posterior mantle. These features are mostly (possibly all) shared with "*Pareledone*" *adeliana* but it differs from the latter by the absence of supraocular papillae in "*P.*" *polymorpha* as well as in colour ("*P.*" *adeliana* is purple-pink to purple-grey dorsally; Lu and Stranks, 1994), and in size ("*P.*" *adeliana* grows to a total length of 160 mm, males mature at 40 mm ML, females at 45 mm ML; Lu and Stranks, 1994).

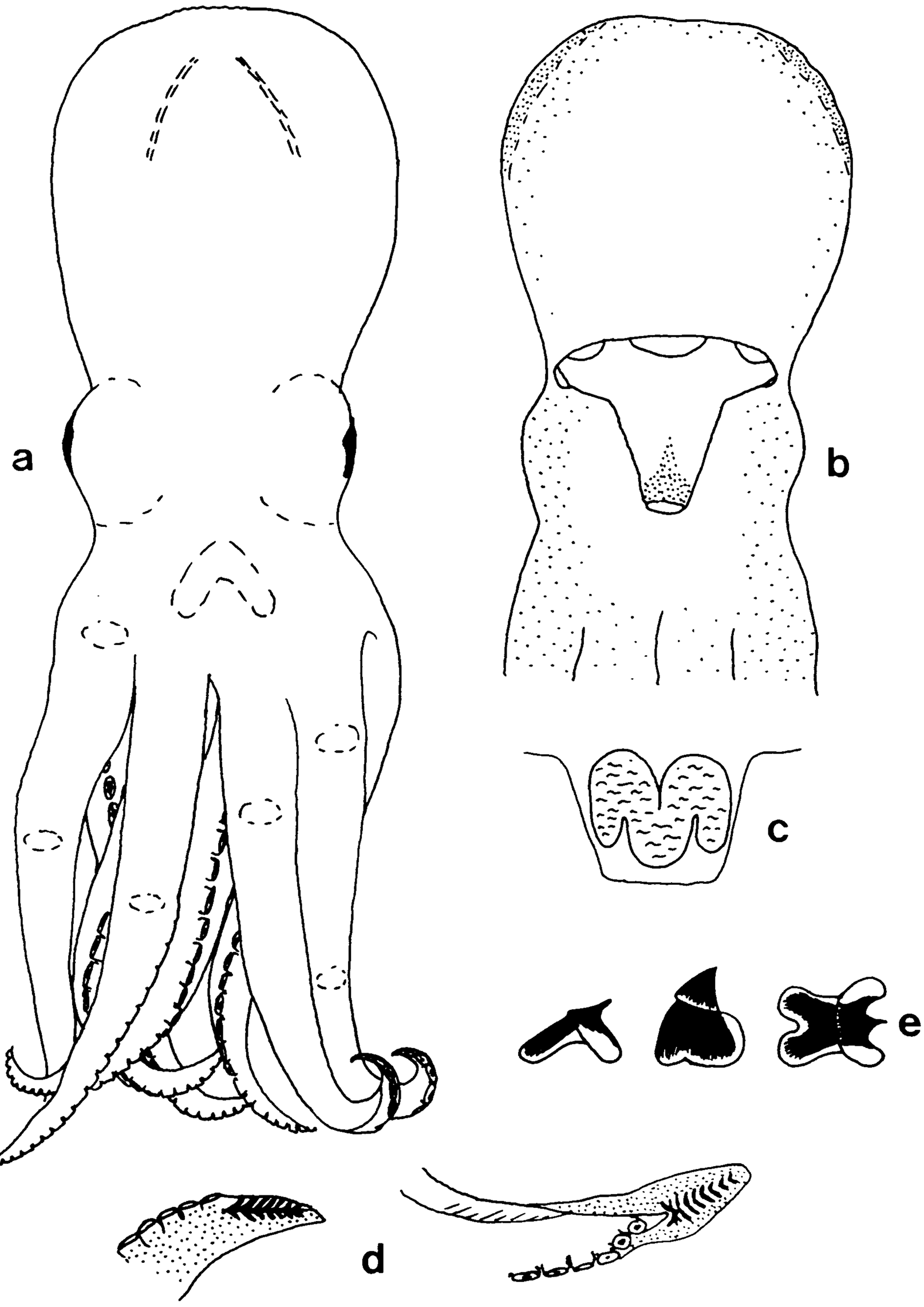


Figure 4.10a-e: "*Pareledone*" *polymorpha*: a, dorsal view of whole animal b, ventral view of mantle and funnel c, funnel organ d. hectocotylus e, beak. All illustrations actual size except beak, twice actual size.

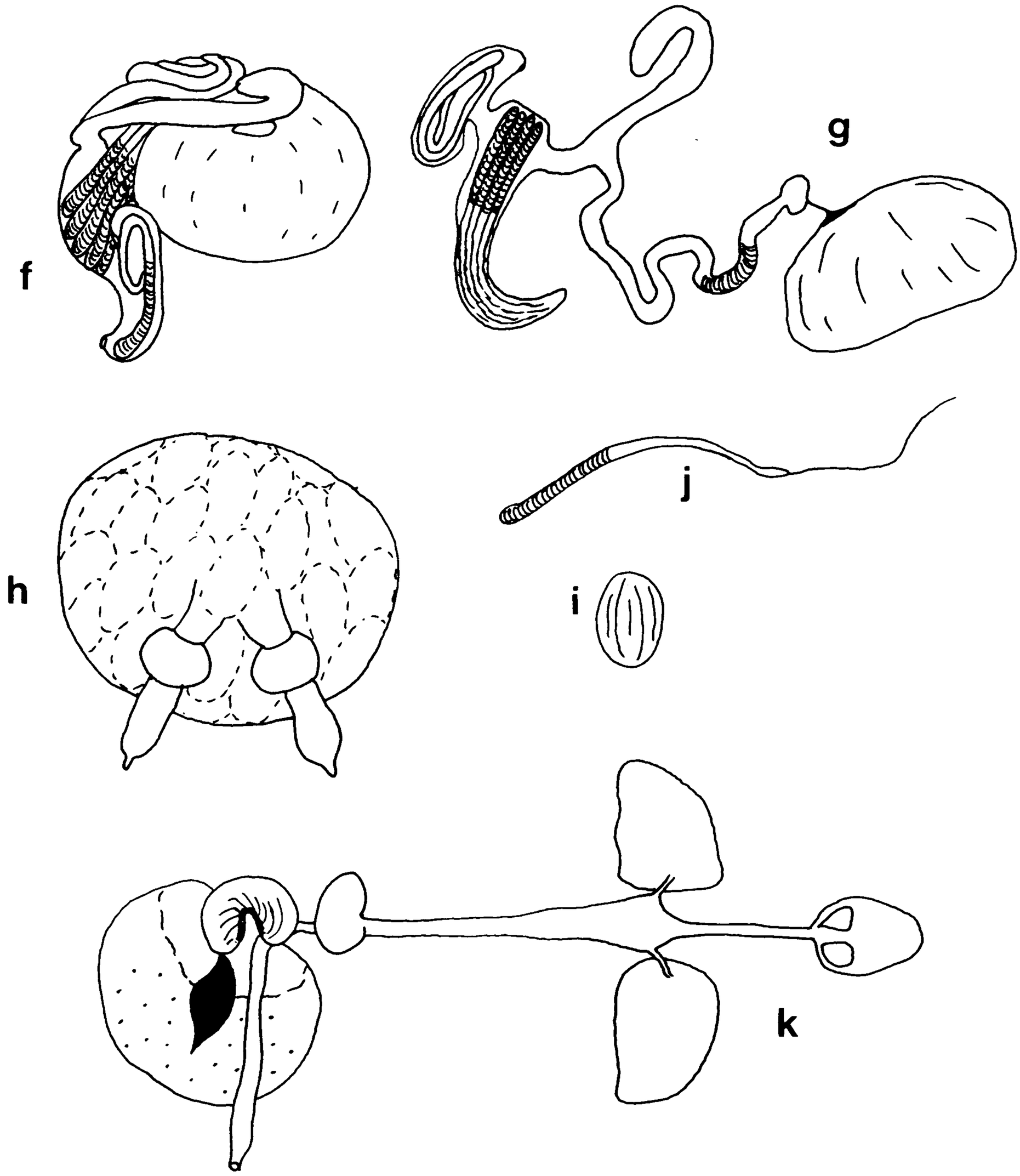


Figure 4.10f-k: *"Pareledone" polymorpha*: f. male reproductive system as positioned in situ g, male reproductive organs dissected out h, female reproductive system i. mature ovarian egg j, mature spermatophore k, digestive system. All illustrations actual size.

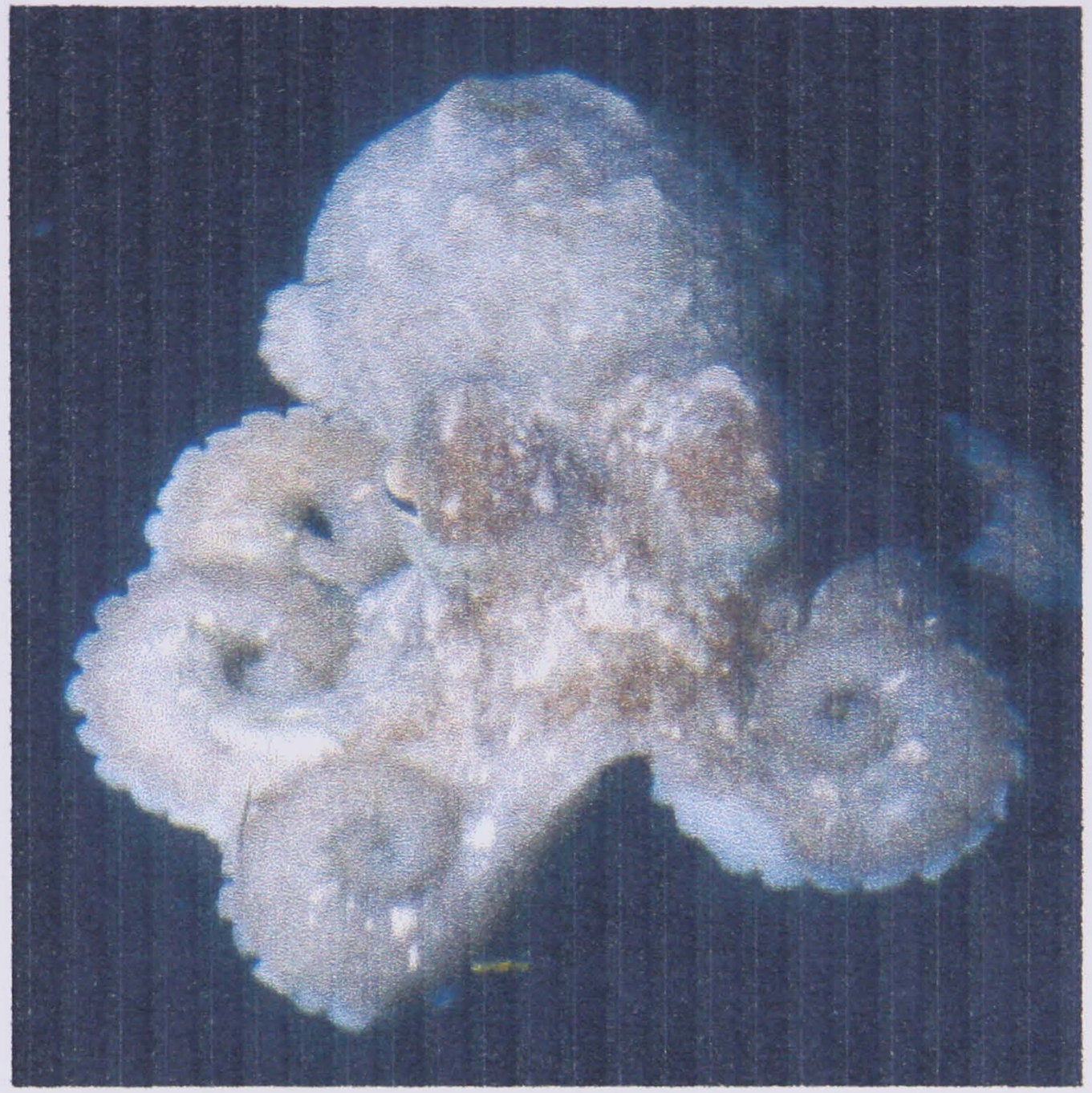


Plate 4.19 "*Pareledone*" *polymorpha*: 53 mm ML.

"*Pareledone*" sp. 18

Figure 4.11

Material examined. **Antarctic Peninsula**, PFS *Polarstern*, 27 November - 19 December 1996. — 61°42'S 59°10'W, 807 m, Stn 42/045: AP1951 (M3, 58 mm ML), AP1952 (M3, 51 mm ML), AP1953 (M3, 52 mm ML), AP1954 (M3, 59 mm ML), AP1956 (F3, 64 mm ML). —61°35'S 58°45'W, 785 m, Stn 42/048: AP2087 (M3, 64 mm ML), AP2088 (M2, 68 mm ML), AP2089 (F2, 68 mm ML), AP2091 (F3, 72 mm ML), AP2092 (F3, 58 mm ML), AP2093 (M3, 60 mm ML), AP2094 (F2, 68 mm ML), AP2095 (F3, 69 mm ML), AP2097 (F3, 73 mm ML), AP2098 (F3, 62 mm ML), AP2099 (F2, 58 mm ML), AP2101 (F2, 60 mm ML), AP2102 (F2, 53 mm ML), AP2103 (F2, 52 mm ML), AP2104 (M2, 60 mm ML), AP2106 (M2, 63 mm ML), AP2107 (M2, 57 mm ML), AP2108 (F3, 58 mm ML), AP2109 (M2, 57 mm ML). —62°15'S 56°57'W, 1514 m, Stn 42/145: AP2403 (F3, 72 mm ML).

Diagnosis. Beak small; rostral tip of lower beak sharp. Ligula groove long, well-marked and deep with transverse ridges. Arms short. Hectocotylised arm with 26-28 suckers. Gills with 6-9 lamellae per demibranch. Integument loose and smooth without papillae. Dorsal and ventral surfaces covered in dense purplish grey chromatophores.

Description. Animals medium sized (ML to 73 mm: TL to 196 mm). Mantle ovoid (MWI 65.5-77.5-91.5) (Figure 4.11a). Eyes medium sized (EDI 20.0-24.7-33.3). Funnel large (Figure 4.11b) (FuLI 26.9-34.0-41.2) gently tapered; funnel organ W-shaped (Figure 4.11c). Arms short (MAI 52.5-63.7-81.0). Arm lengths subequal, arm order usually 3=4.2.1 (ALI L1 93.8-141.4-177.4; L2 107.8-148.2-190.3; L3 114.8-152.0-181.1; L4 118-152.4-182.4). Suckers uniserial, small (ASI 3.5-5.1-6.8), without sucker enlargement. Third right arm of males hectocotylised, shorter than opposite number (OAI 87.1-95.0-103.1). Ligula large (LLI 13.1-14.0-14.9); ligula groove long, well marked and deep, with transverse ridges. Calamus distinct (Figure 4.11d). Hectocotylised arm with 26-28 suckers, opposite arm with 36-44 suckers. Web of medium depth (WDI 22.1-24.5-29.2), web formula usually B=C=D.A=E. Ink sac reduced. Chromatophores on dorsal surface of digestive gland. Posterior

salivary glands large (Figure 4.11k). Gills with 6-9 lamellae per demibranch (mode: inner gill lamellae count 8, outer gill lamellae count 7.) Beak small; rostral tip of lower beak sharp (Figure 4.11e). Mature ovarian eggs large, 16 mm x 9 mm (EgLI 14.5-20.5-27.6; EgWI 8.1-10.2-15.5) (Figures 4.11h, i). Spermatophores shorter (SpLI 54.2-63.2-73.1), slender (SpWI 5.3-5.8-6.2) (Figures 4.11f, g, j). Males mature at approximately 60-65 mm ML, females at approximately 60-70 mm ML.

The integument is smooth and loose. There are no papillae. Two short, longitudinal integumentary ridges are present on the mid-dorsal posterior mantle. There is no ventrolateral integumentary ridge. All surfaces are completely covered in purplish grey chromatophores, except two areas around the funnel that are white (Figure 4.11b).

Distribution. Antarctic Peninsula [61°35'-62°15'S 56°57'-59°10'W] (this study). The recorded depth range of this species is 570-1523 m.

Remarks. This species shares with "*Pareledone*" *polymorpha* a W-shaped funnel organ, chromatophores on the dorsal surface of the digestive gland, a small sharp beak, large posterior salivary glands, considerably shorter spermatophores, a large ligula with transverse ridges and two short longitudinal integumentary ridges on the mid-dorsal posterior mantle. These features distinguish it from other species of *Pareledone* except "*P.*" *polymorpha* and "*P.*" *adeliana*. It may be distinguished from both "*P.*" *polymorpha* and "*P.*" *adeliana* by the presence of densely packed chromatophores on the ventral surface.

An unusual feature of this species is the apparent regression of the ink sac. This was studied in detail in twelve specimens. In five of these it was not possible to detect an ink sac at all, although this does not necessarily deny its presence as ink sacs in this species are buried deep within the digestive gland and are remarkably difficult to locate. In five specimens the ink sac was located, removed and measured. In all cases the ink sac was less than 6 mm in length (excluding duct) and less than 1

mm in width. In two specimens a duct was located but this did not apparently enlarge to form a sac.

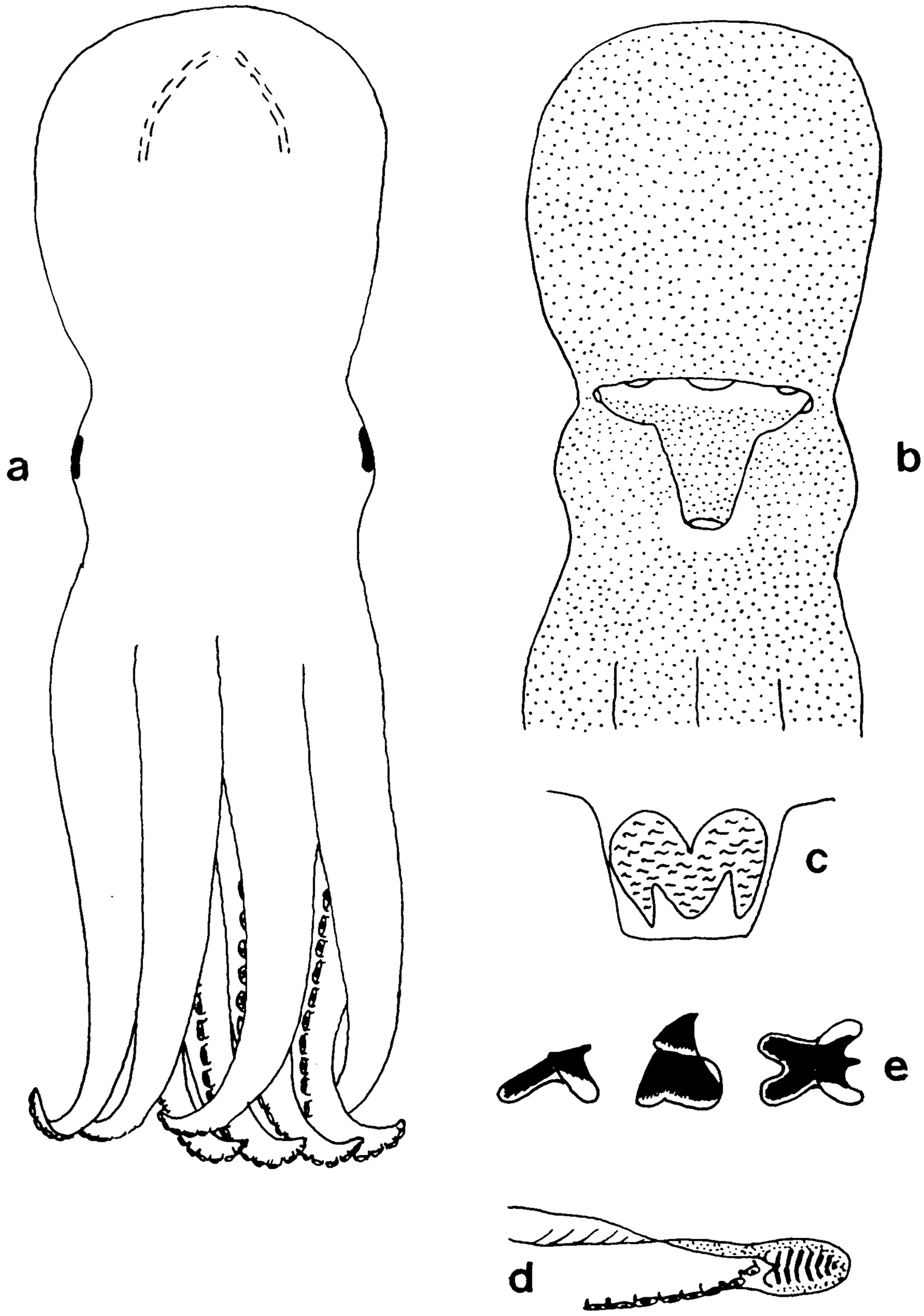


Figure 4.11a-e: "*Pareledone*" sp 18: a, dorsal view of whole animal b, ventral view of mantle and funnel c, funnel organ d, hectocotylus e, beak. All illustrations actual size except beak, twice actual size.

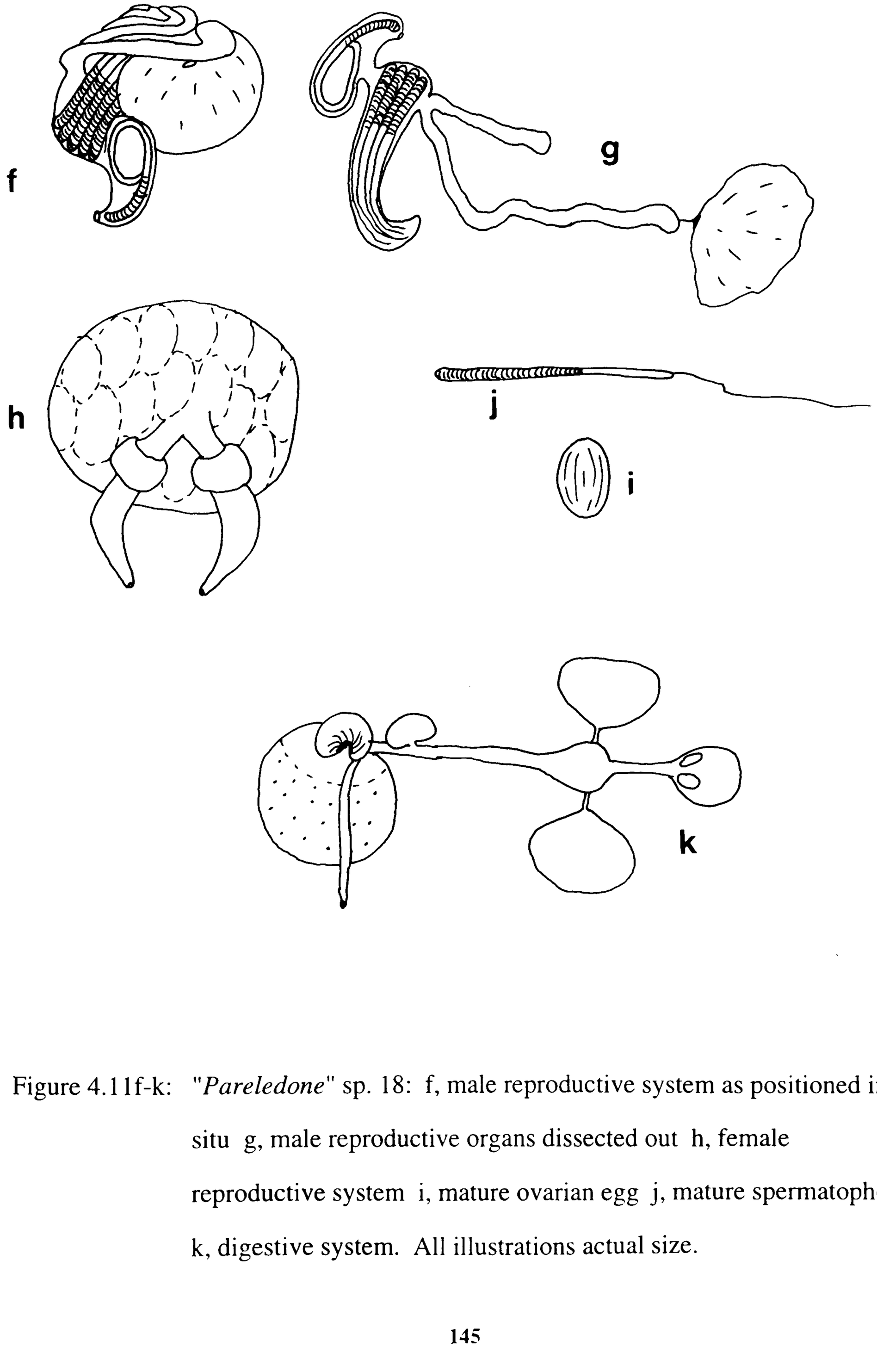


Figure 4.11f-k: "*Pareledone*" sp. 18: f, male reproductive system as positioned in situ g, male reproductive organs dissected out h, female reproductive system i, mature ovarian egg j, mature spermatophore k, digestive system. All illustrations actual size.

"*Pareledone*" sp. 28

Material examined. **Antarctic Peninsula**, PFS *Polarstern*, 26 December 1996. —66°36'S 68°42'W, 607m, Stn 42/077: AP2463 (M3, 68 mm ML), AP2464 (F1, 51 mm ML).

Diagnosis. Beak small; rostral tip of lower beak sharp. Ligula groove long, well-marked and deep with transverse ridges. Arms medium length. Hectocotylised arm with 31 suckers. Gills with 7-9 lamellae per demibranch. Ventral surface covered in irridescent green markings.

Description. Animals medium sized (ML to 68 mm: TL to 182 mm). Mantle ovoid (MWI 79.4-81.9-84.3). Eyes medium sized (EDI 25.0-25.2-25.5). Funnel large (FuLI 33.3-35.8-38.2) gently tapered; funnel organ W-shaped. Arms medium length (MAI 53.7-54.5-55.3). Arm lengths approximately equal. Suckers uniserial, small (ASI 3.9-4.2-4.4), without sucker enlargement. Third right arm of males hectocotylised, shorter than opposite number (OAI 92.7). Ligula medium sized (LLI 7.9); ligula groove long, well marked and deep, with transverse ridges. Calamus distinct. Hectocotylised arm with 31 suckers, opposite arm with 42-45 suckers. Web shallow (WDI 17.1-17.5-17.9). Gills with 7-9 lamellae per demibranch.

The integument is smooth and loose; it is wrinkled above the eyes. There are no obvious papillae. Two short, longitudinal integumentary ridges are present on the mid-dorsal posterior mantle. A ventrolateral integumentary ridge is also present. Brown chromatophores completely cover both dorsal and ventral surfaces. The ventral surface is also covered in iridescent green markings.

Distribution. Only known from the above locality.

Remarks. This species apparently shares those features that separate "*Pareledone*" from *Pareledone* but more specimens are required to describe this species fully.

Discussion

The diagnosis of *Pareledone* given by Lu and Stranks (1994) was broader than that given here. It did not give a description of the form of the ligula, nor did it include the size and shape of spermatophores, or a description of the beak. Furthermore, it allowed VV- or W-shaped funnel organs. These differences allowed "*P.*" *adeliana* to be included in the genus *Pareledone*. The many and consistent differences between "*P.*" *adeliana*, "*P.*" *polymorpha*, "*P.*" sp. 28, "*P.*" sp. 18 and all other species of *Pareledone* are strong evidence that these four species should be moved to a separate new genus.

The diagnosis of this new genus should be as follows. Benthic octopodids. Mantle saccular, without fins. Eight arms lacking cirri, arms with small uniserial suckers, third right arm of males hectocotylised with end of arm clearly differentiated into ligula and calamus, ligula large, ligula groove long, well marked and deep, with transverse ridges, arm tips not otherwise modified. Web shallow to medium. Funnel organ W-shaped. Gills well developed with 6-9 lamellae. Ink sac present or reduced. Crop well developed, posterior salivary glands large. Cartilaginous stylets absent. Two short, longitudinal integumentary ridges on the mid-dorsal posterior mantle. Spermatophores short and slender. Beak small, rostral tip of lower beak sharp.

Daly (1996) commented on a possible connection between the large posterior salivary glands exhibited by members of this genus and the small delicate beak. She suggested "*Pareledone*" *polymorpha* may partially digest prey before ingestion and that this practice may have led to the partial regression of the beak because of its reduced function. She also suggested that the beak of "*P.*" *polymorpha* may be specialised to exploit a non-benthic food source, such as euphausiids in the water column directly above the sea bed.

Of the four species that would comprise this new genus, three are reported only from Western Antarctica whilst the fourth, "*P.*" *adeliana*, was thought, until recently, to be confined to Eastern Antarctica. Kubodera and Okutani (1994) report

specimens of "*P.*" *adeliana* from the Antarctic Peninsula, however their description states that there are "no distinct papillae over eyes", whereas the description of Lu and Stranks (1994) records the presence of a "large supraocular papilla". In Robson's (1930) original description of "*P.*" *polymorpha* there is no mention of supraocular papillae and none were found on specimens taken from the type locality during this study. It is therefore possible, that the specimens of Kubodera and Okutani (1994) are in fact "*P.*" *polymorpha* and "*P.*" *adeliana* is indeed confined to Eastern Antarctica.

The reduction of the ink sac in "*Pareledone*" sp. 18 is an extremely unusual feature. Some specimens appeared to completely lack an ink sac, and this would, under accepted schemes of classification, place them in a separate subfamily. There is so much consistency between other characters, however, there is no doubt that this species belongs in the same genus as "*P.*" *polymorpha*. The implications of these findings are discussed more fully in Chapter 7.

Although it appears necessary to split four species from the genus *Pareledone*, a large genus still remains. It currently contains twelve species, eight of which have dense papillae covering at least their dorsal surface, four of which have only fine, scattered papillae. The smoother skinned species may also be distinguished by the number of gill lamellae: they have 8-11 lamellae as opposed to 6-9 in those species with dense papillae. Allozyme electrophoresis also indicates that the smoother skinned species are genetically distant from those with dense papillae (see Chapter 2). Although this is a clear division, all twelve species have many other features in common (e.g., size and shape of spermatophores, structure of hectocotylus, shape of beak etc.) and it does not appear necessary to split the genus any further.

The eight papillose species can be easily distinguished, either by the structure of their papillae or by the arrangement of enlarged papillae. Distinguishing between the smoother skinned species is more difficult. Although *P.* sp. 14 sp. nov. has a slightly higher gill lamellae count there is an overlap with the range of other species.

In fact, the ranges of most characters overlap between these four species (e.g., HSC: 31-45 in *P. turqueti*, 34-40 in *P.* sp. 13 sp. nov., 31-38 in *P.* sp. 14 sp. nov., 36-50 in *P. harrissoni*) and, although a computer could correctly classify the majority of specimens when given information from a large number of characters (see Chapter 3), in reality the only truly useful character for distinguishing between the smoother skinned species (apart from biochemical and molecular characters; see Chapter 2) is the integumental sculpture of the live animal. It is possible, therefore, that specimens of *P. turqueti* and *P. harrissoni*, that have only been studied by octopus taxonomists after fixation, have been incorrectly classified and these are unlikely ever to be classified with complete confidence. Voss (1988b) declared *P. harrissoni* a *nomen dubium*. He gave no reasons for this statement but since the type specimen is extant (Lu and Stranks, 1994), and the original description is fuller and clearer than many others given at the time (Berry, 1917) it cannot be because satisfactory typification is impossible. It must therefore be because he feels it is "impossible to ascertain to which taxon [the] type should be referred" (Jeffrey, 1989). Voss' statement is perhaps understandable considering the overlap of measurable characters between this species and *P. turqueti*.

With the uncertainty regarding the identity of previous specimens it is almost impossible to assess the range of these two species, but it is probably better to assume that *P. harrissoni* is confined to Eastern Antarctica and *P. turqueti* to Western Antarctica until there is new evidence to the contrary.

The large number of new species described recently (nine since 1994) is mostly a reflection of the fact that there had been no concentration of research effort in this field since the 1920s. Only small samples had been available (20 specimens would constitute a large haul) and mostly the researchers concerned were not afforded the opportunity of viewing the animals live. Observations of live animals is important in octopus systematics (e.g., Hanlon and Hixon, 1980; Hanlon, 1988; Roper and Hochberg, 1988) and this has proved especially so in the case of the *Pareledone*.

Two species which look quite similar when dead, but are very distinctive when alive are *Pareledone charcoti* and *Pareledone* sp. 16 sp. nov.. The unique papillae of the latter often seem to 'relax' when the animal is dead and show a greater resemblance to the papillae of *P. charcoti*. In formalin preserved specimens the structure of the papillae would presumably depend upon how long a specimen of *P.* sp. 16 sp. nov. had been dead before it was preserved. In the early part of this century there was some confusion over subsequent descriptions of *P. charcoti* and Berry (1917) erected a new but similar species *P. aurorae*. In 1918, Berry concluded his species was synonymous with *P. charcoti* after publication of new figures of the latter species by Joubin (1914). Since many of the specimens from this period were taken from the Antarctic Peninsula, and often from shallow water where *P.* sp. 16 sp. nov. is dominant, one wonders if, in fact, researchers were studying specimens of both *P. charcoti* and *P.* sp. 16 sp. nov.. This is, of course, pure speculation. If possible, however, the specimens of Joubin, Hoyle and Berry from the early part of this century should be re-examined alongside the recently collected specimens of *P. charcoti* and *P.* sp. 16 sp. nov. from off the Antarctic Peninsula, when the latter arrive in the UK.

Finally, the eggs seen in all the species of *Pareledone* are large. Octopuses with large eggs usually produce benthic hatchlings which have a limited dispersal range (Hochberg *et al.*, 1992). This fits in with the situation seen in the Antarctic, where there are many species, each apparently confined to a small area. This is discussed in greater depth in Chapters 6 and 7.

Chapter 5

The distribution of Octopodidae captured in the western sector of the Southern Ocean during this study.

Introduction

The specimens captured during this research undoubtedly represent the largest collection of Antarctic octopodids ever studied; the specimens possibly number more than the sum of all other collections. Commercial bottom trawls were deployed at 145 stations in the Western Southern Ocean, and an Agassiz net was deployed at a further 29 stations. The sampled depth range was also large (from 70 m to 3,219 m depth) although some depths were sampled more thoroughly than others. Sampling opportunities often arose through surveys on commercial fish species and this has resulted in an imbalance in fishing depths; shallower depths (100-400 m) were studied extensively, whilst some depths were not investigated at all.

The aim of this chapter is to map the capture locations of the octopodid species encountered in Western Antarctica during this study, and also to try to assess how well various areas have been sampled, and to what extent we are likely to have discovered all the extant species of octopuses in the region.

Methods

Field Sampling

Between 4th January and 8th February 1994 the waters around South Georgia and Shag Rocks were surveyed by the Falkland Islands' fishery patrol vessel *MV Cordella* using a commercial bottom trawl as part of an annual survey of demersal finfish stocks in the area. The same stations are fished most years and in previous years three species of octopus had occurred as by-catch in the trawls. Although stations had been selected as far as possible at random, fishing was not possible off part of the southern coast of South Georgia where uneven ground meant that damage to the trawl gear was inevitable. 98 stations were fished in total, the majority of which were 100-300 m depth; five stations were 300-400 m depth (Table 5.1). The sampling yielded 707 octopodid specimens, comprising three species in two subfamilies.

Between 5th and 28th February 1996 a benthic survey of the coastal shelf of the Weddell Sea was undertaken by the German research vessel *PFS Polarstern*. Five gear types (commercial bottom trawl, benthopelagic trawl, Agassiz trawl, box-corer and epibenthic sled) were deployed off Kapp Norvegia and between Vestkapp and Halley Bay. Only the bottom trawl and the Agassiz trawl were thought to be suitable for capturing octopodids. The epibenthic sled and box-corer caught one specimen each, however, because of problems with the net sounder, the benthopelagic trawl touched the bottom on one occasion and large numbers of octopodids were found in the net. The majority of trawling took place in depths less than 400 m (Table 5.1). The sampling yielded 447 octopodid specimens comprising eight species in two subfamilies.

Between 16th November and 26th December 1996 a benthic survey of the Antarctic Peninsula was undertaken by *PFS Polarstern*. Three gear types (commercial bottom trawl, benthopelagic trawl and Agassiz trawl) were deployed off Elephant Island, King George Island, and Adelaide Island. Recurrent problems with the net sounder meant that the benthopelagic net occasionally touched the sea floor and hence yielded benthic octopodids. A demersal finfish survey during the cruise meant that trawling was extensive in depths less than 400 m (Table 5.1). Cruise time was, however, also dedicated to cephalopod work (using the bottom and benthopelagic trawls) and isopod / amphipod work (using the Agassiz trawl) and this allowed some deeper areas to be surveyed (Table 5.1). The sampling yielded 2,464 specimens comprising twenty-one species in three subfamilies.

Between October 1992 and January 1995 fishery observers aboard commercial vessels licensed to fish in the Falkland Islands 200 mile zone collected octopodids from the bycatch. Although most of the octopodid specimens came from commercial bottom trawls in depths of less than 400 m, two octopus specimens were also taken from long lines at 1,000 m depth (Table 5.1). The sampling yielded 138 specimens comprising five species in four subfamilies.

Technical and biological data from these surveys were compiled and stored in a computer database.

Data Analysis

To assess the likelihood that the survey around the Antarctic Peninsula was comprehensive enough to have discovered all the species in that area, the cumulative number of species found at various depth ranges was plotted against the number of trawls. Plots such as these produce a curve that approaches an asymptote at a y-value corresponding to the maximum number of species in the area. Clearly for these curves to be meaningful, each trawl must sample the same unit area of ground, hence the gear that had been fished most extensively at any one depth was selected to examine that depth. The depth ranges were chosen arbitrarily to fit with the fishing strategies rather than for biological reasons. Once gear type and towing time (i.e., the time the gear spends on the sea floor sampling the benthos) had been selected for each depth (Table 5.2) data from all the trawls that fitted these criteria were extracted from the database. The shape of the curve, and hence the point at which the asymptote is reached, is dependent upon the order in which trawls are plotted. The order of the trawls was therefore randomised three times using a computerised random number generator and the cumulative number of species was plotted against the number of trawls three times on the same axis.

Map Production

Maps showing capture locations for each species encountered during the surveys were plotted. Although some maps were drawn by hand, the majority were produced using British Antarctic Survey's Geographic Information System (GIS). This holds cartographic information on the Antarctic coastlines and digital bathymetric data on the Southern Ocean. Hence the GIS allows the rapid production of computer generated charts. The octopodid data stored in the GIS to produce these charts is now available to other researchers using the system.

Table 5.1: Fishing strategies employed during the surveys. Numbers in parentheses indicate number of trawls that yielded specimens. CBT, commercial bottom trawl; AGT, Agassiz trawl; BPN, benthopelagic trawl; LL, long line. There are no records for stations that did not yield octopodids in the Falkland Islands.

General location	Gear	Number of times gear deployed at depths			
		<400 m	400-600 m	600-1,000 m	>1,000 m
South Georgia	CBT	90 (82)			
Weddell Sea	CBT	3 (3)	5 (5)	2 (2)	
	AGT	5 (4)	1 (0)	1 (0)	2 (0)
	BPN		1 (1)		
Antarctic Peninsula	CBT	38 (38)	3 (3)	4 (4)	
	AGT	4 (3)	8 (8)		8 (3)
	BPN	2 (0)	4 (3)	10 (0)	1 (0)
Falkland Islands	CBT	(56)			
	LL				(2)

Table 5.2: Gear type and towing time selected to test how comprehensively various depth ranges off the Antarctic Peninsula were surveyed for octopodids.

Depth	Gear	Towing time	No. of trawls
100-400 m	Commercial bottom trawl	30 mins	32
400-600 m	Agassiz trawl	15 mins	8
600-800 m	Commercial bottom trawl	60 mins	4
>1,500 m	Agassiz trawl	40 mins	8

Results

Assessing the efficiency of the sampling programme

Although sampling was extensive on the Antarctic Peninsula, the majority of trawls were deployed in shallow (<400 m bottom depth) waters (Table 5.1). When cumulative number of species is plotted against the number of trawls deployed at these depths (Figure 5.1a), the resultant curve appears to reach an asymptote, suggesting that samples of all species that occur in these depths on the Antarctic Peninsula may have been obtained.

At 400-600 m depth eight species were yielded by Agassiz trawls (Figure 5.1b). There is no evidence from the curve that eight is likely to be the maximum number of species at these depths; in fact, additional tows using a commercial bottom trawl and a benthopelagic net (which touched the bottom) (Table 5.1) yielded four additional species. It is not possible to assess whether twelve species are all that occur in these depths as data from the three gear types cannot be combined.

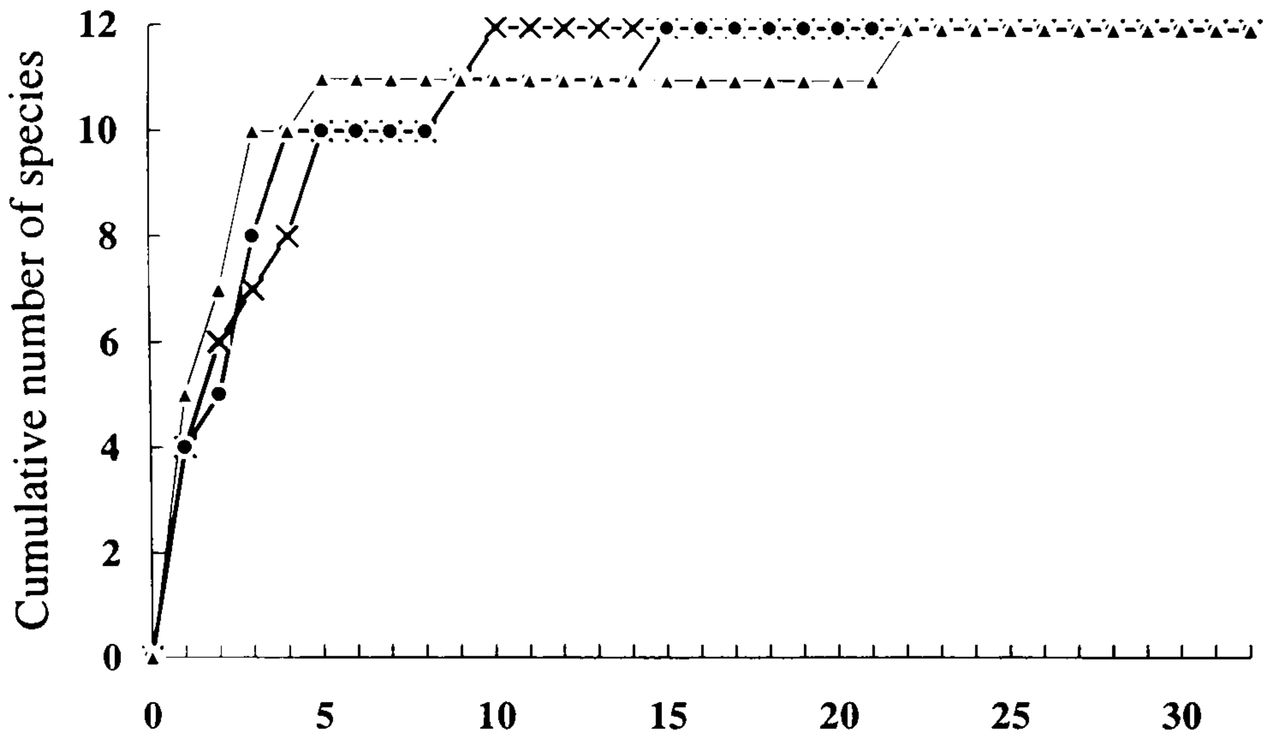
Nine species were captured at 600-800 m depth (Figure 5.1c). Although the plot of cumulative number of species against the number of trawls flattens off slightly, it appears unlikely that specimens of all the species that occur at these depths have been captured.

Six species were captured at depths below 1,500 m. There is no evidence that the plot of cumulative number of species against the number of trawls is tending towards an asymptote, and it is highly likely that there are species present at these depths that were not discovered during this survey.

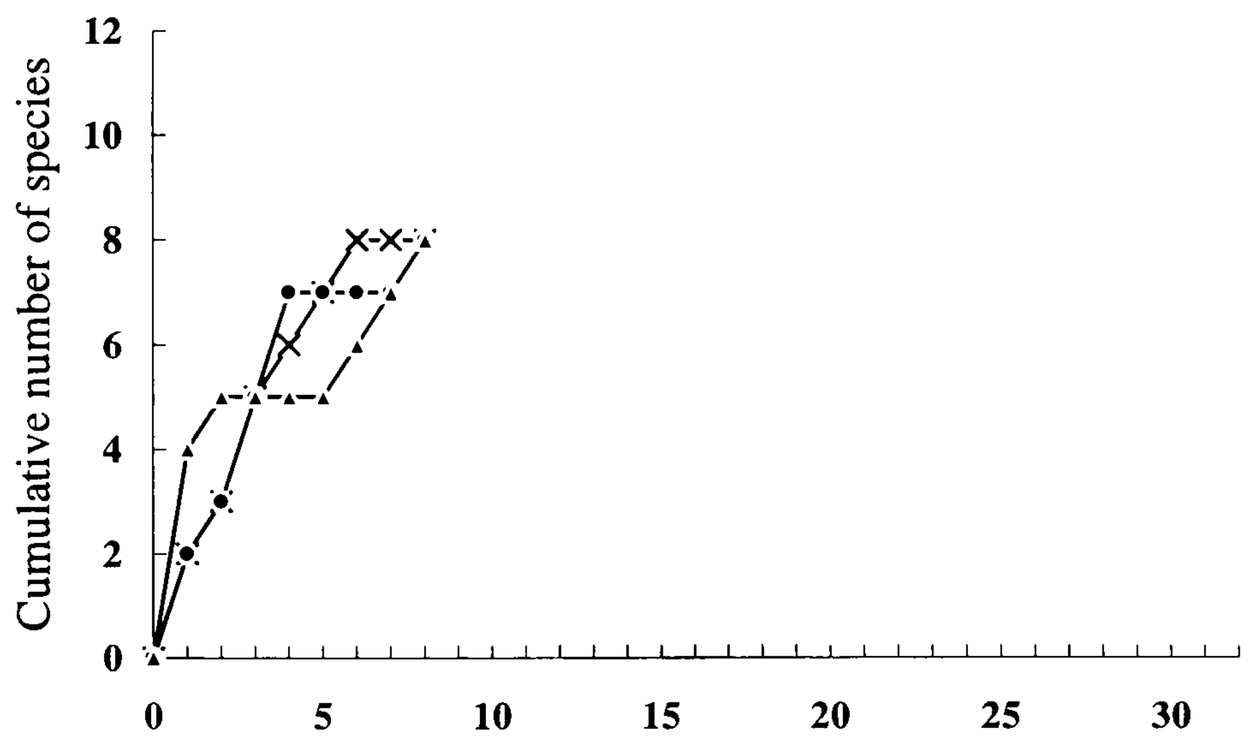
Recorded distributions of Octopodidae in the Southern Ocean

1. Eledoninae

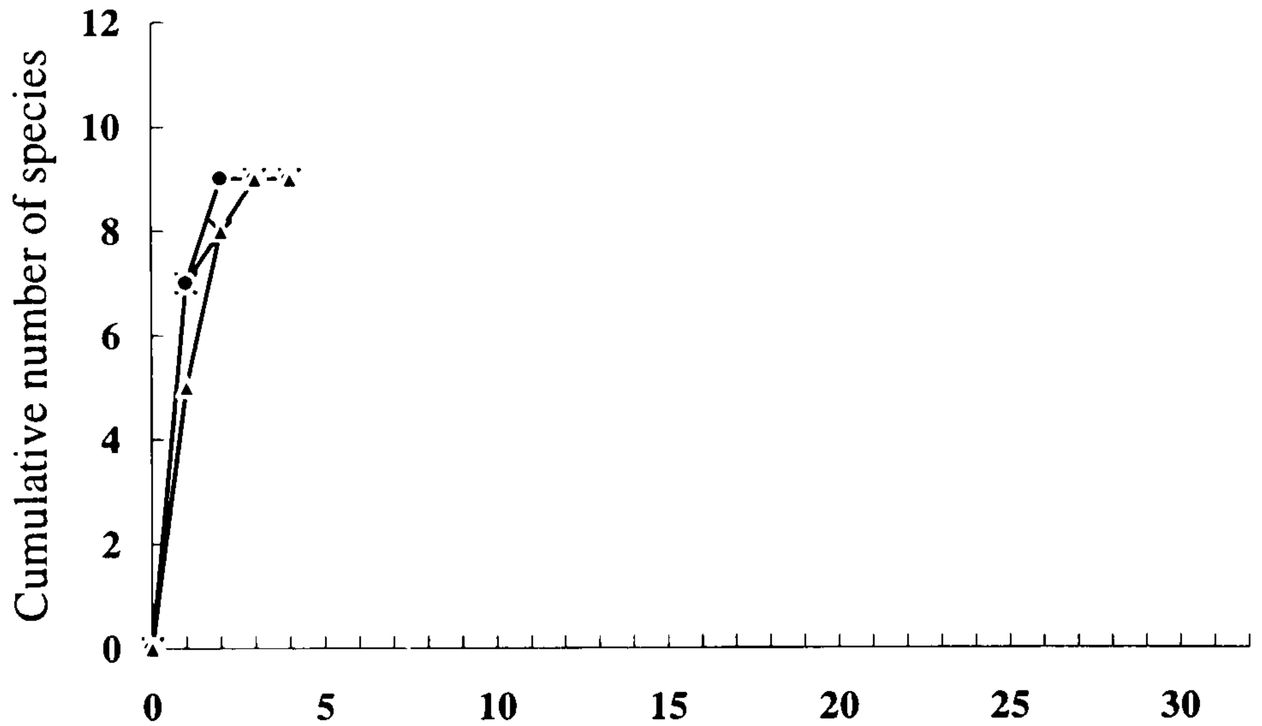
Sixteen, possibly seventeen (depending on the status of *Pareledone* sp. 27: see Chapters 2 and 7), species of the subfamily Eledoninae were captured from the Western Southern Ocean. The subfamily appears to be represented at all depths down to 1,500 m, although depth ranges for individual species vary greatly (Figure



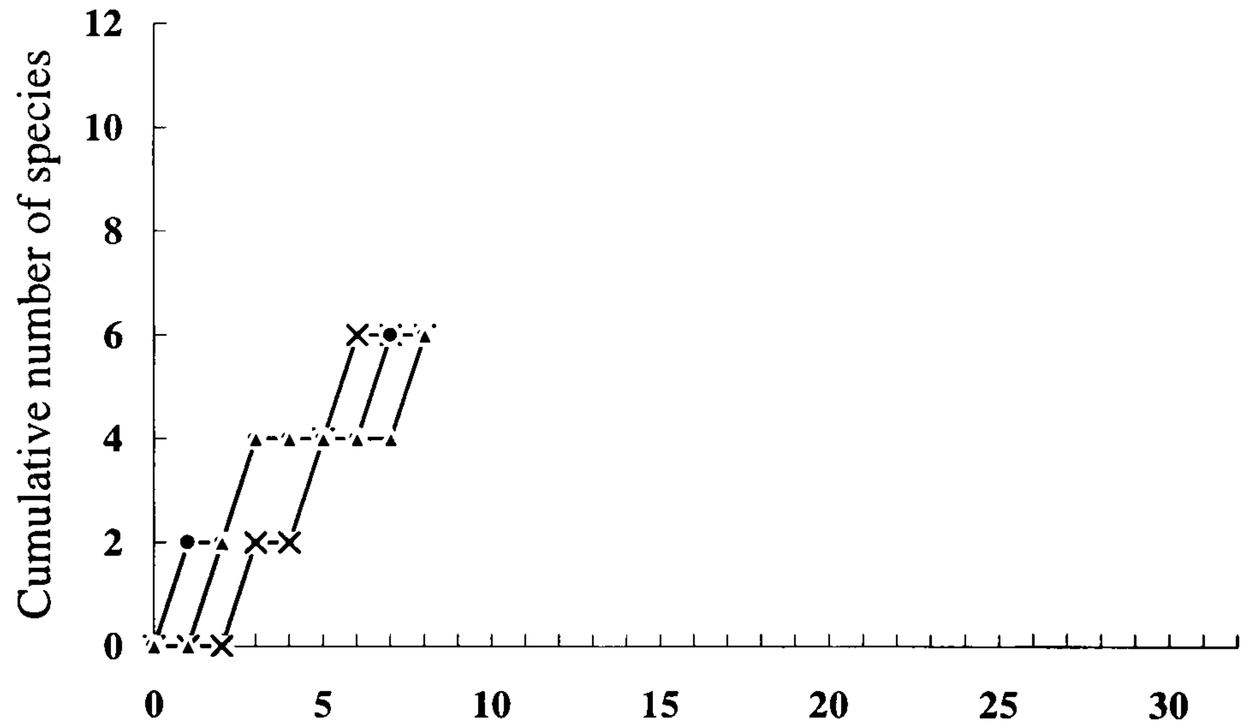
a) Number of trawls (100-400 m depth)



b) Number of trawls (400-600 m depth)



c) Number of trawls (600-800 m depth)



d) Number of trawls (>1,500 m depth)

Figure 5.1: Assessing the efficiency of the sampling programme. Plots of cumulative number of species against number of trawls for four depth ranges off the Antarctic Peninsula. Trawls ordered randomly and plotted three times.

5.2). Capture locations for each species of Eledoninae are illustrated in Figures 5.3-5.15. *Pareledone turqueti* (Figure 5.3) and "*Pareledone*" *polymorpha* (Figure 5.13) were found at all three of the Western Antarctic locations sampled in this study (South Georgia, Weddell Sea, Antarctic Peninsula): *Pareledone charcoti* (Figure 5.4) and *Megaleledone senoi* (Figure 5.12) were found at two (Weddell Sea, Antarctic Peninsula). All other species of the subfamily Eledoninae were found only at a single location.

2. Graneledoninae

Eight putative species of the subfamily Graneledoninae were captured. Only four could be identified to species level, but various authors (Stranks *et al.*, in prep; Vecchione *et al.*, in prep) are currently tackling the taxonomic problems provided by these specimens. Three specimens of *Thaumeledone gunteri* were captured at a single station off South Georgia at 364-394 m depth (Figure 5.16). This was the second deepest station off South Georgia and it is therefore likely more specimens could be captured if the fishing effort were concentrated differently. The other seven species were captured from the Antarctic Peninsula where a greater emphasis was placed on deeper trawling (Table 5.1); they were captured from depths ranging from 386 m to below 3,000 m (Table 5.3, Figure 5.17).

3. Bathypolypodinae

Benthoctopus ?levis was the only species captured that belongs to the subfamily Bathypolypodinae. It was found both in the Weddell Sea and off the Antarctic Peninsula (Figure 5.18) in depths ranging from 208 m to 889 m.

Recorded distributions of Octopodidae around the Falkland Islands

Once it was established that the fauna north of the Antarctic Polar Front was different to that found in the Southern Ocean, little attention was devoted to the octopuses of the Falkland Islands. In this thesis they have been used mainly as

Genus	Species	(n)	Depth range
Pareledone	*turqueti*	545	
	charcoti	864	
	sp. 11 sp. nov.	44	
	sp. 10 sp. nov.	40	
	sp. 13 sp. nov.	181	
	sp. 14 sp. nov.	49	
	sp. 15 sp. nov.	42	
	sp. 16 sp. nov.	372	(thin line reflects relative scarcity at these depths)
	sp. 19 sp. nov.	400	
	sp. 26	4	
	sp. 27†	1	
"Pareledone"	*polymorpha*	613	
	sp. 18	47	
	sp. 28	2	(mud haul)
Megaleledone	*senoi*	76	
Not known	sp. 17		
	sp. 22		

0 m 500 m 1,000 m 1,500 m

Figure 5.2: Depth ranges of species of the subfamily Eledoninae recorded from South Georgia, the Weddell Sea and the Antarctic Peninsula. Bottom trawling was not conducted below 900 m depth or above 70 m depth. The range of species is therefore uncertain beyond these limits (indicated by dotted lines). Further limited information was gained from deep water Agassiz tows. (n), number of specimens captured; †, considered conspecific with *Pareledone* sp. 14 sp. nov. in Chapter 2, but see also Chapter 7.

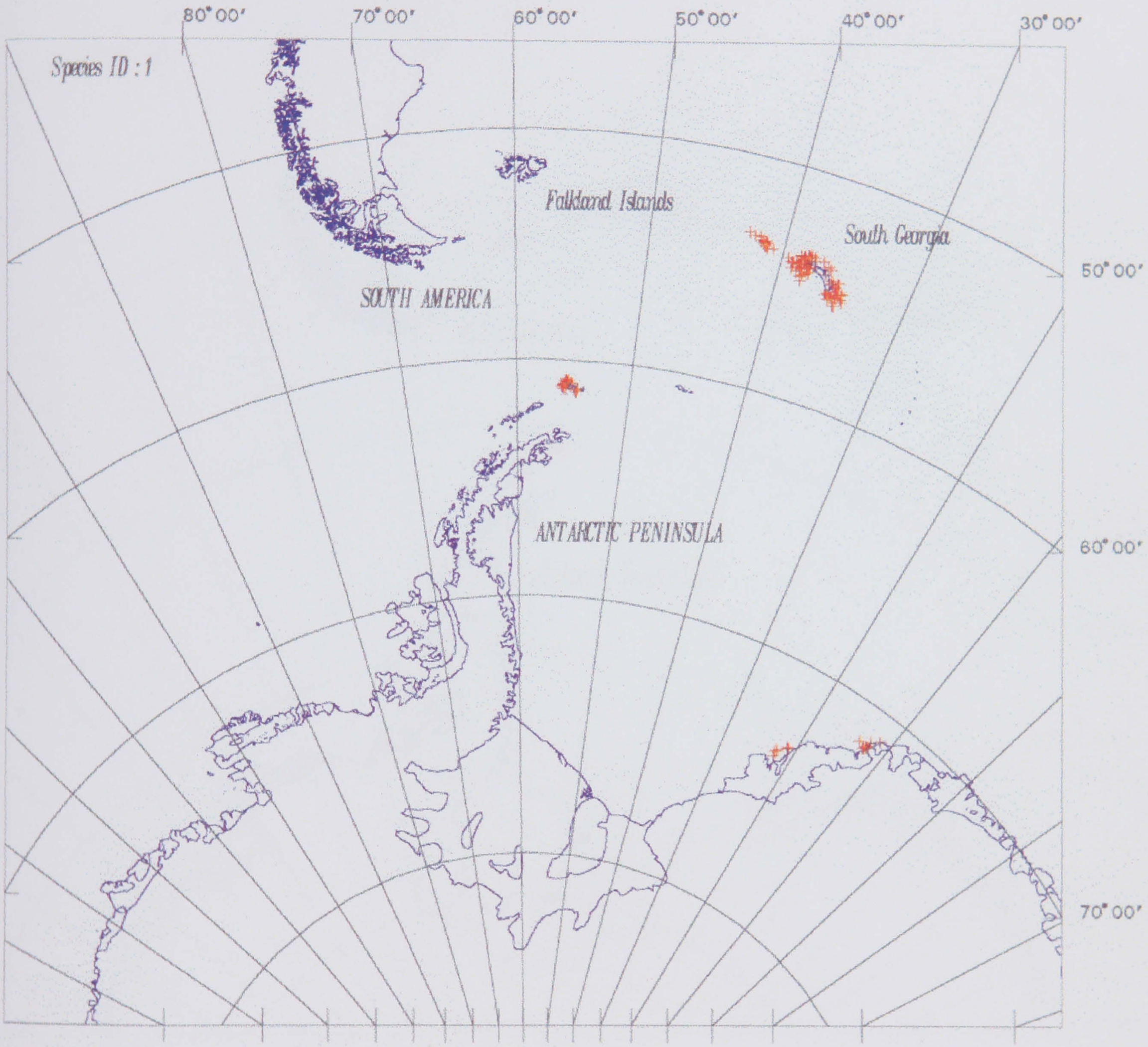


Figure 5.3: *Pareledone turqueti*. **Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.**

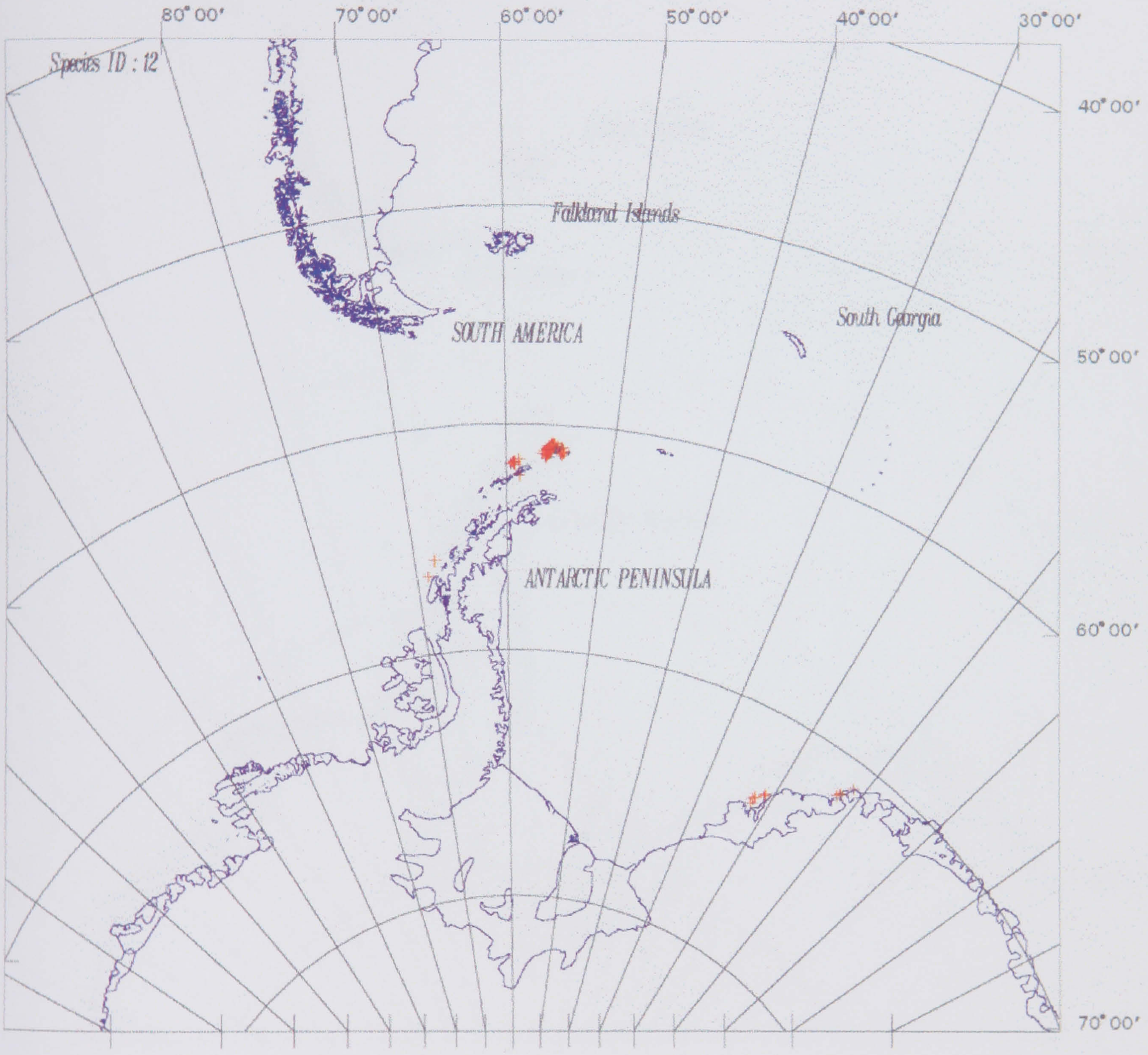


Figure 5.4: *Pareledone charcoti*. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

Figure 5.5: *Pareledone* sp.11 sp. nov.. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

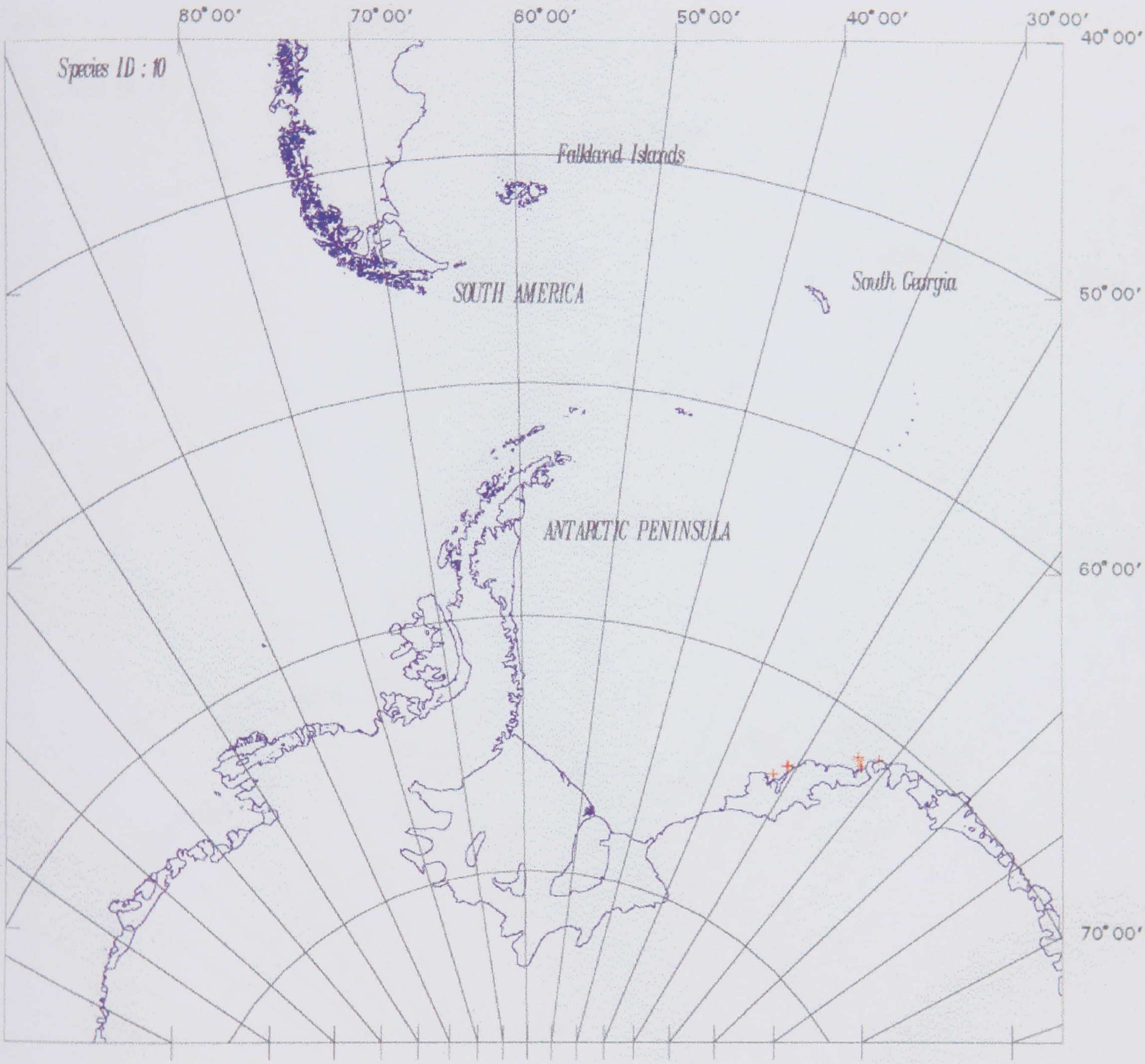


Figure 5.6: *Pareledone* sp. 10 sp. nov.. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

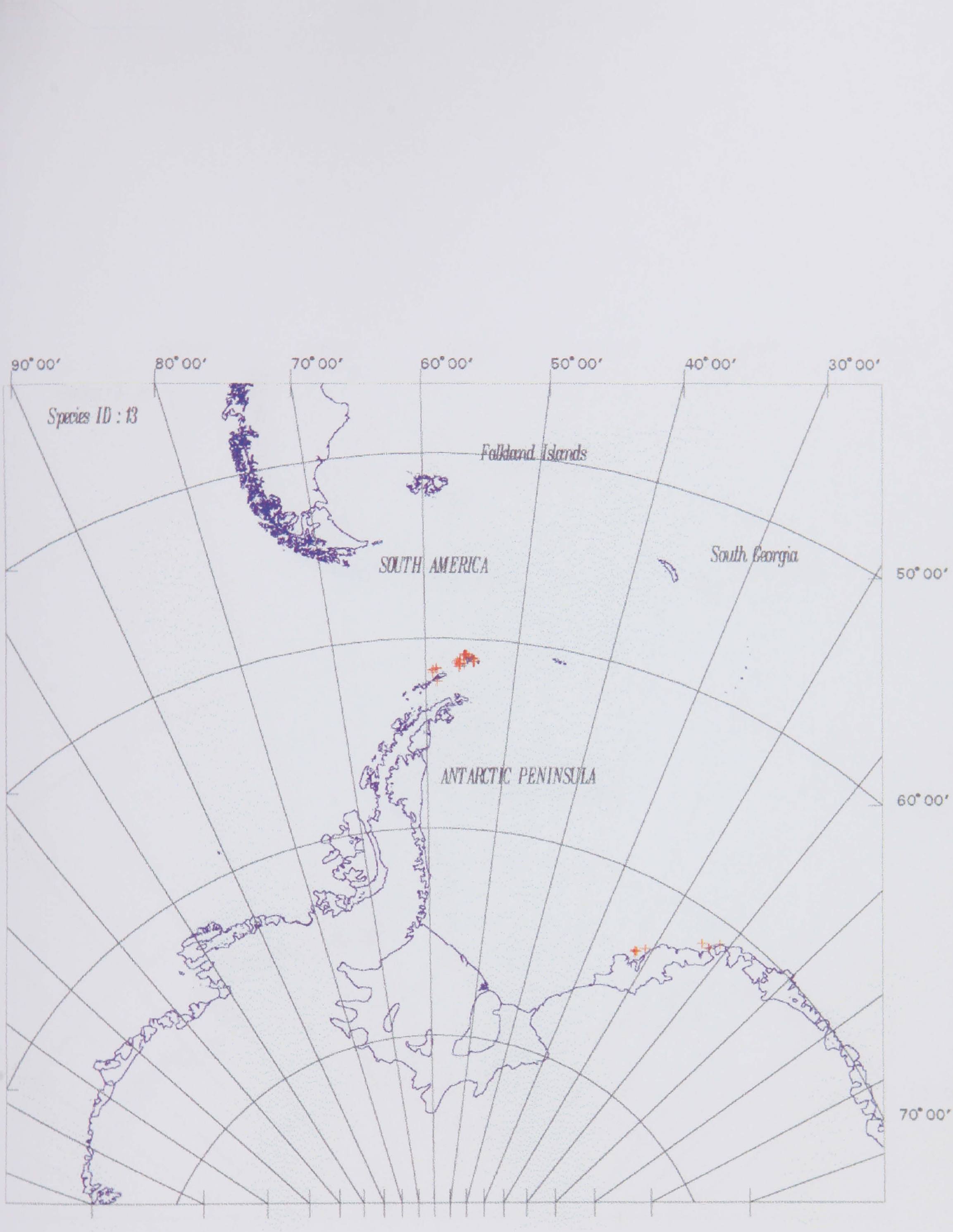


Figure 5.7: *Pareledone* sp. 13 sp. nov.. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

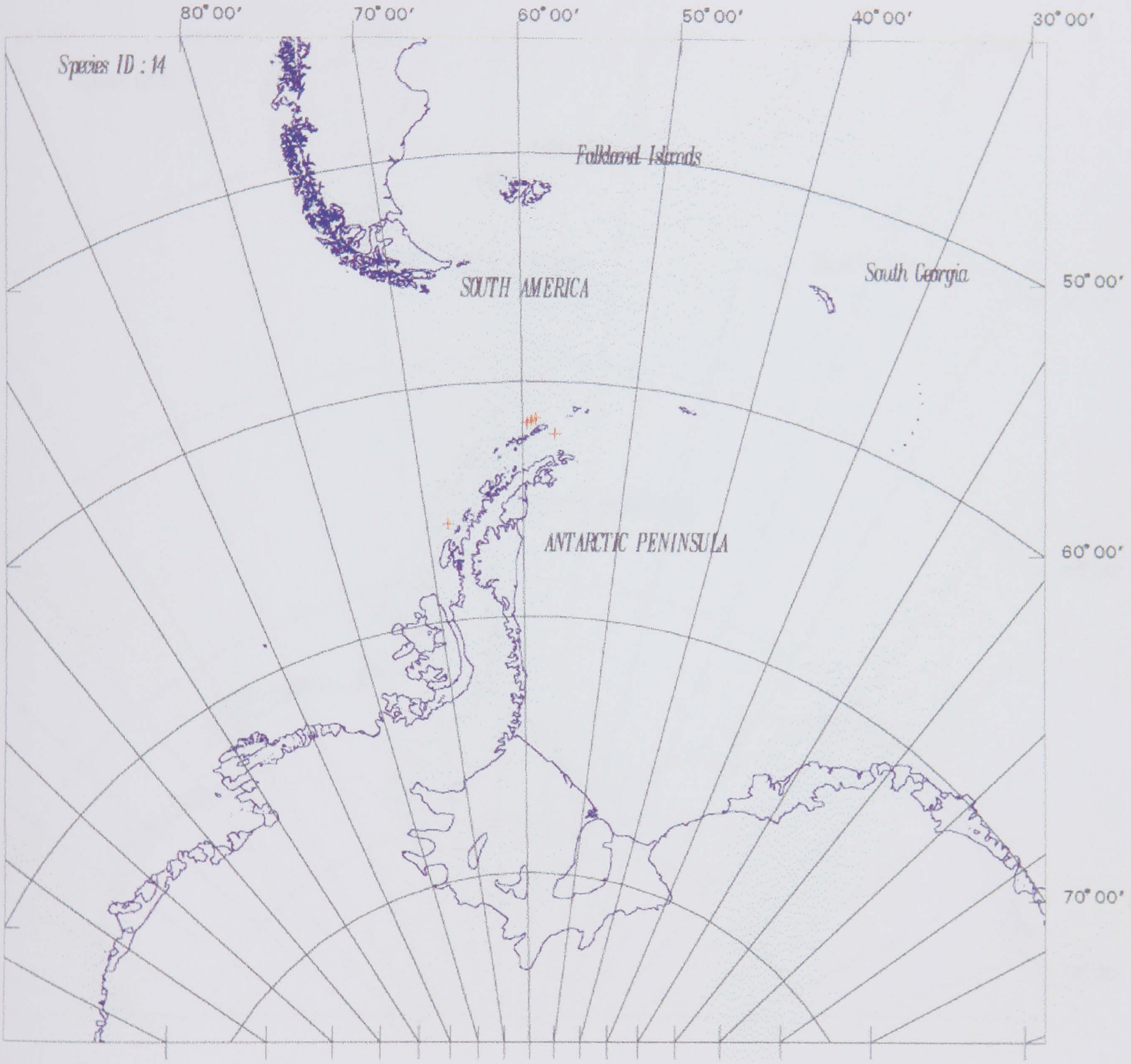


Figure 5.8: *Pareledone* sp. 14 sp. nov.. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

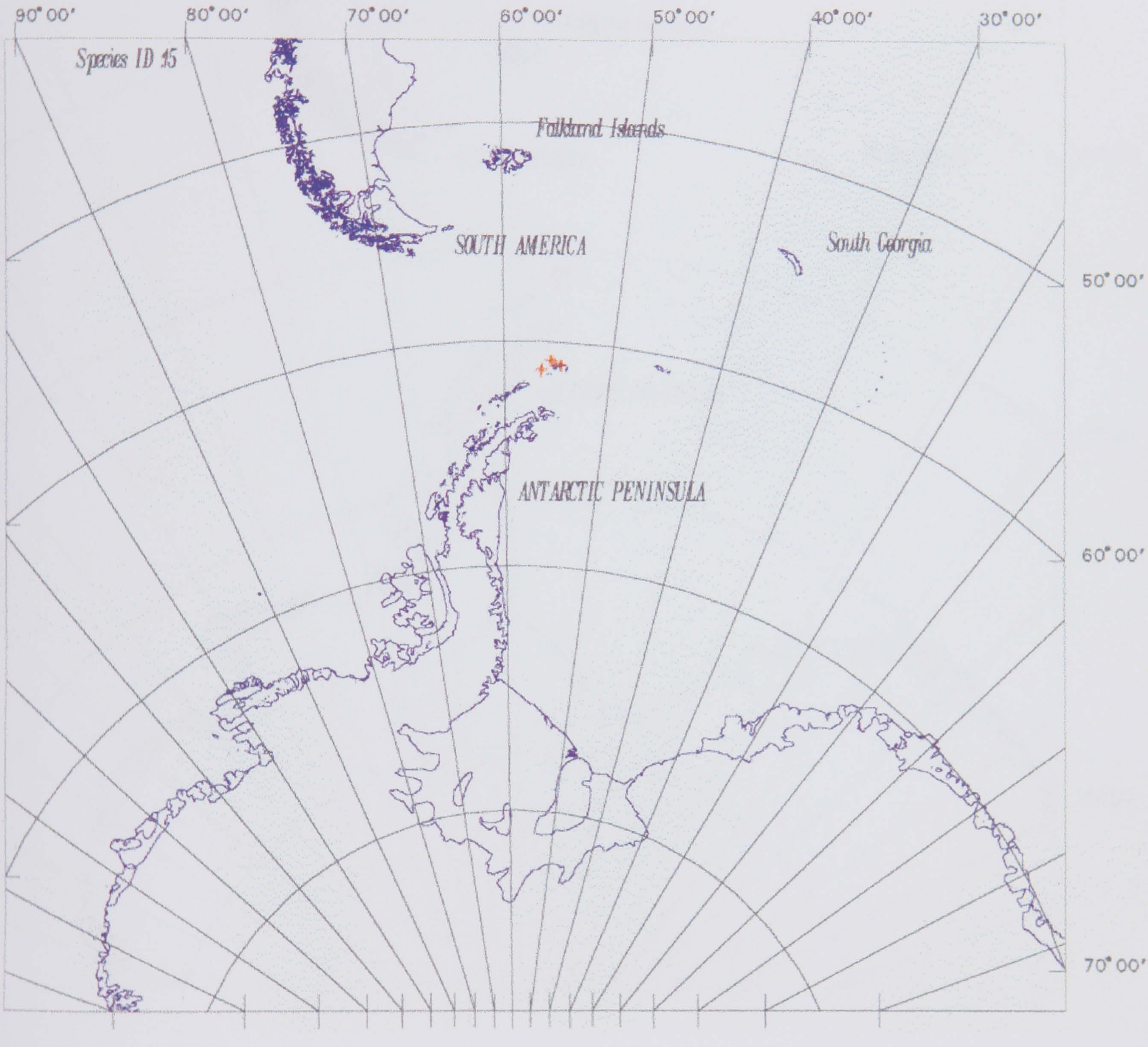


Figure 5.9: *Pareledone* sp. 15 sp. nov.. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

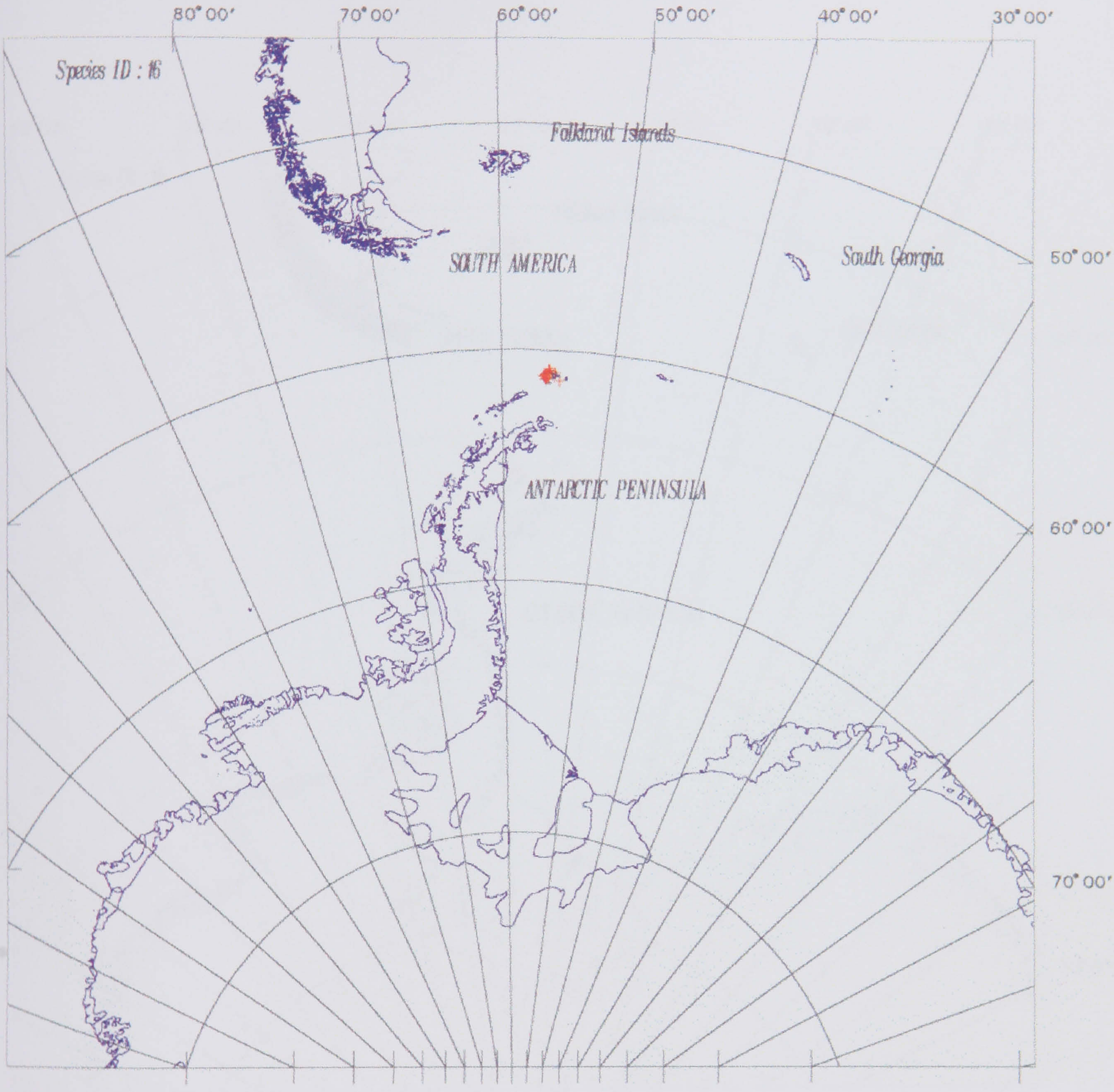


Figure 5.10: *Pareledone* sp. 16 sp. nov.. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

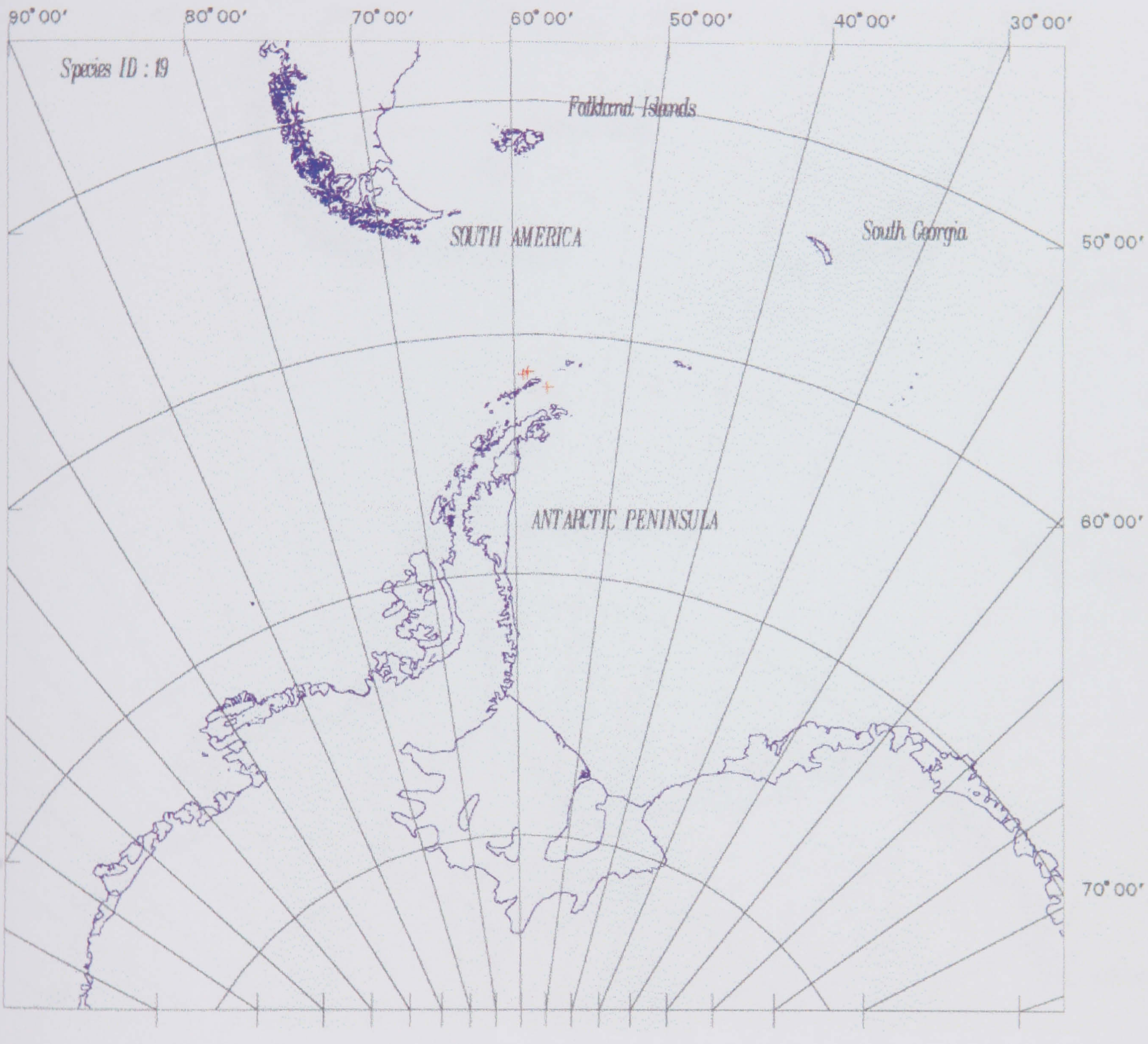


Figure 5.11: *Pareledone* sp. 19 sp. nov.. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

Figure 5.12: *Megaleledone senoi*. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

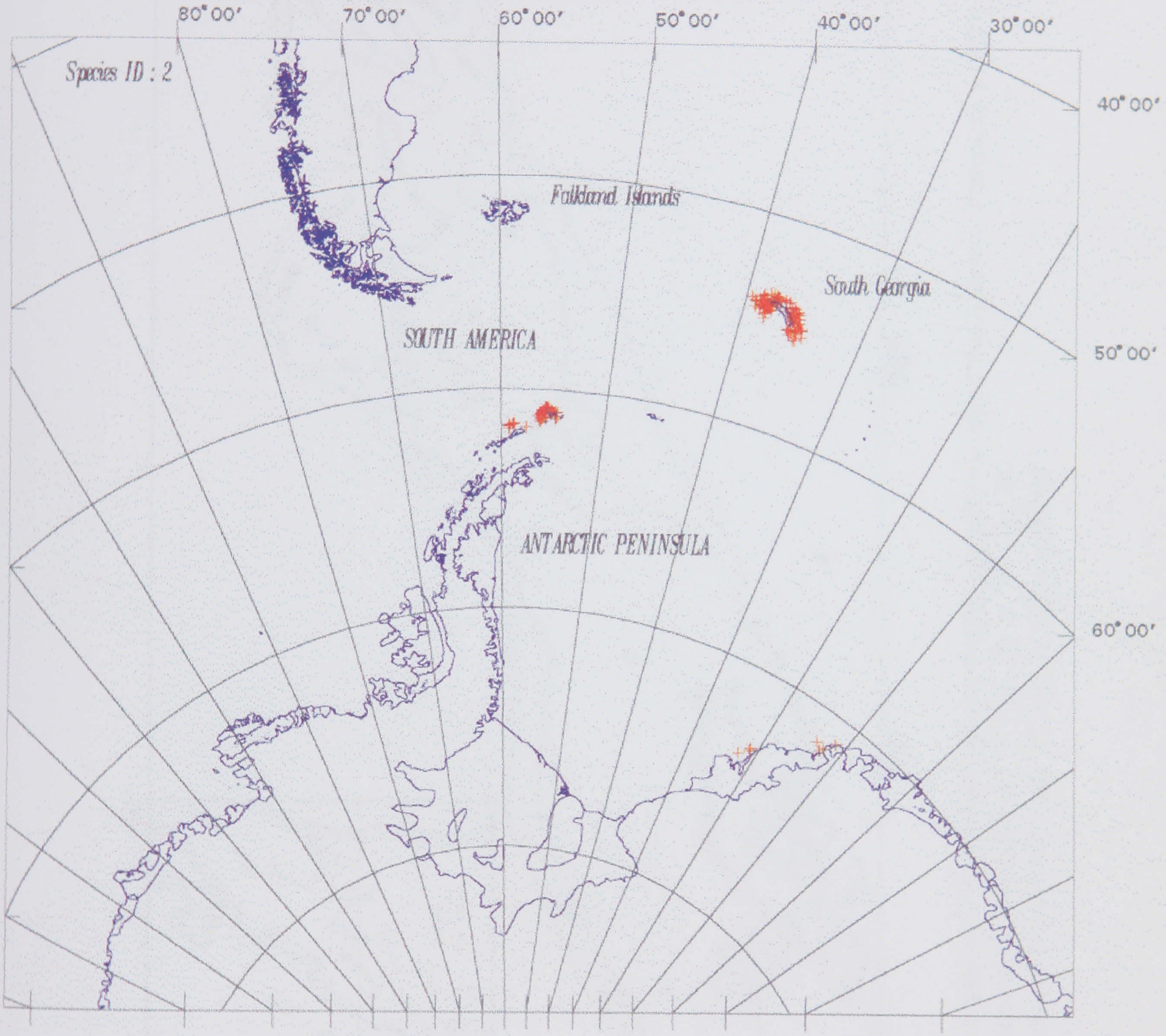


Figure 5.13: "*Pareledone*" *polymorpha*. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

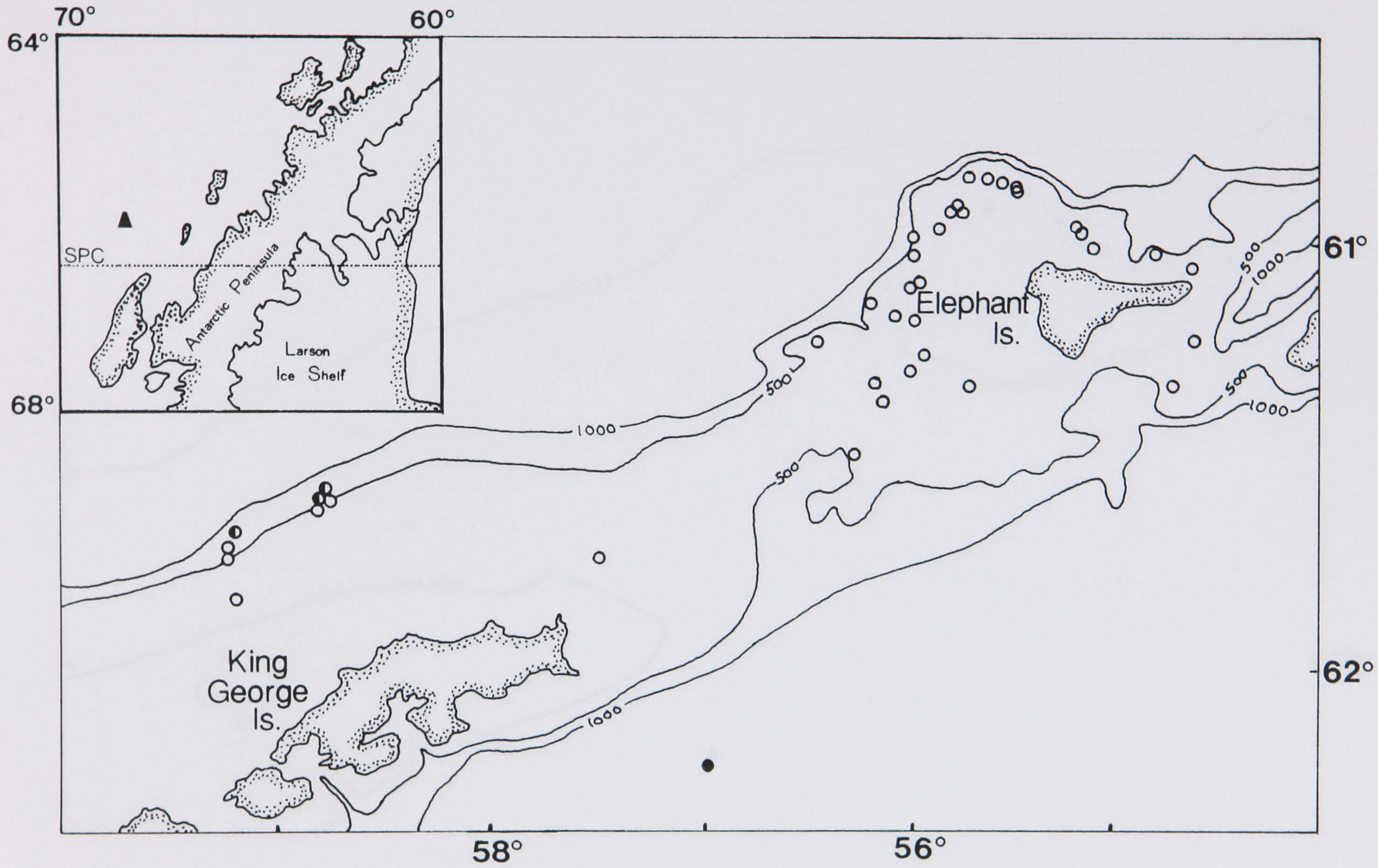


Figure 5.14: Capture locations of "*Pareledone*" species during a benthic survey of the Antarctic Peninsula by *PFS Polarstern*.
o, "*Pareledone*" *polymorpha*; ●, "*P.*" sp. 18; ▲, "*P.*" sp. 28.
◐, "*Pareledone*" *polymorpha* and "*P.*" sp. 18 occur together.

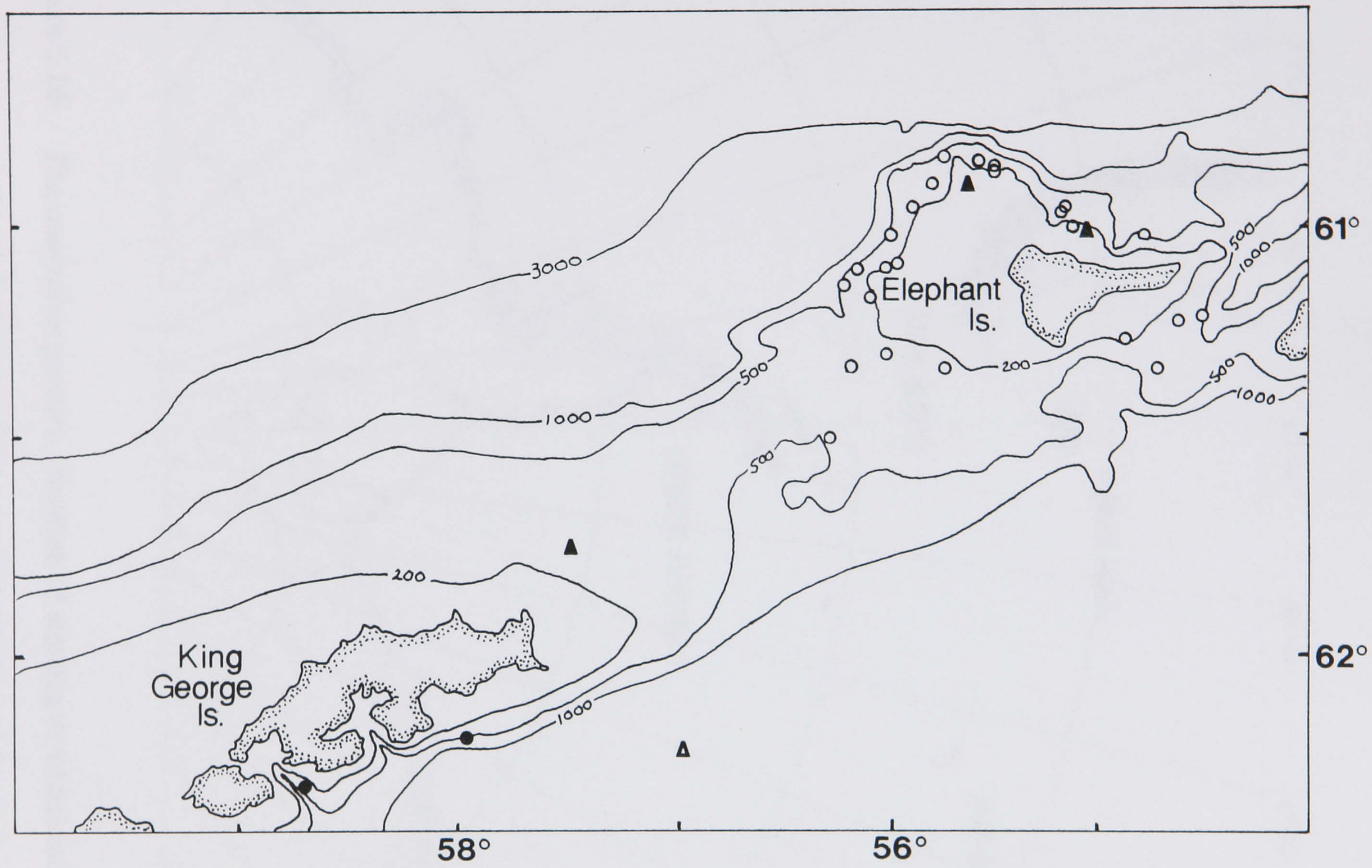


Figure 5.15: Capture locations of four unnamed species of the subfamily Eledoninae during a benthic survey of the Antarctic Peninsula by *PFS Polarstern*. o, sp. 17; ●, sp. 22; ▲, sp. 26; ▲, sp. 27. For discussion of the status of sp. 27 see Chapters 2 and 7.

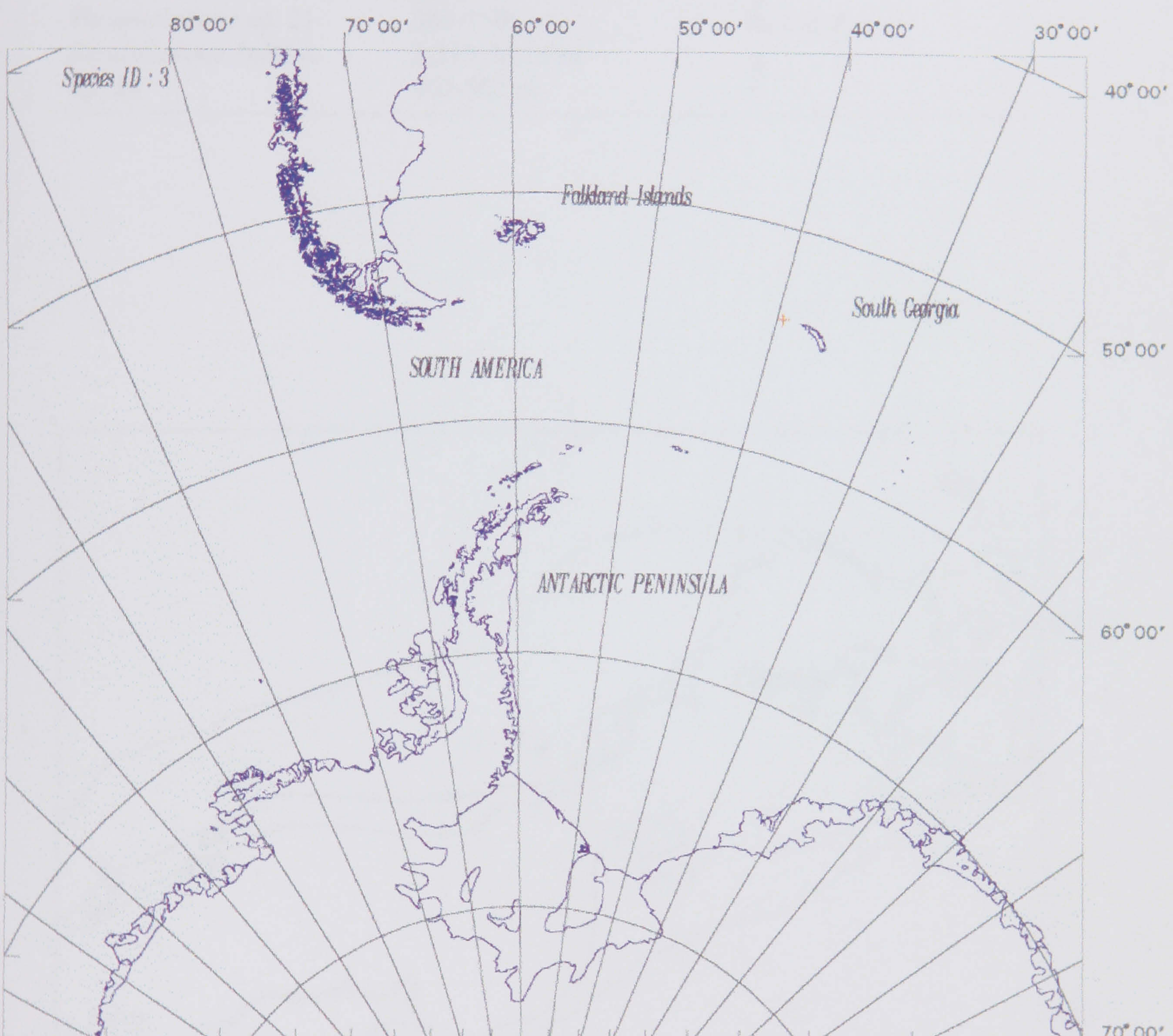


Figure 5.16: *Thaumeledone gunteri*. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

Table 5.3: Capture locations of Graneledoninae off the Antarctic Peninsula during November 1996 - January 1997. For more information on capture locations refer to Figure 5.17.

Species	Recorded depth range	Capture locations
? Graneledone sp. 25	463-503 m	i
? Graneledone sp. 29	491-879 m	a, b, d, e, h
Graneledone antarctica	1,444-1,506 m	f
Thaumeledone ?brevis	3,213-3,219 m	g
Thaumeledone sp. 21	386-1506 m	b, c, e, f
Bentheledone ?albida	3,213-3,219 m	g
Sp. 24	463-503 m	i

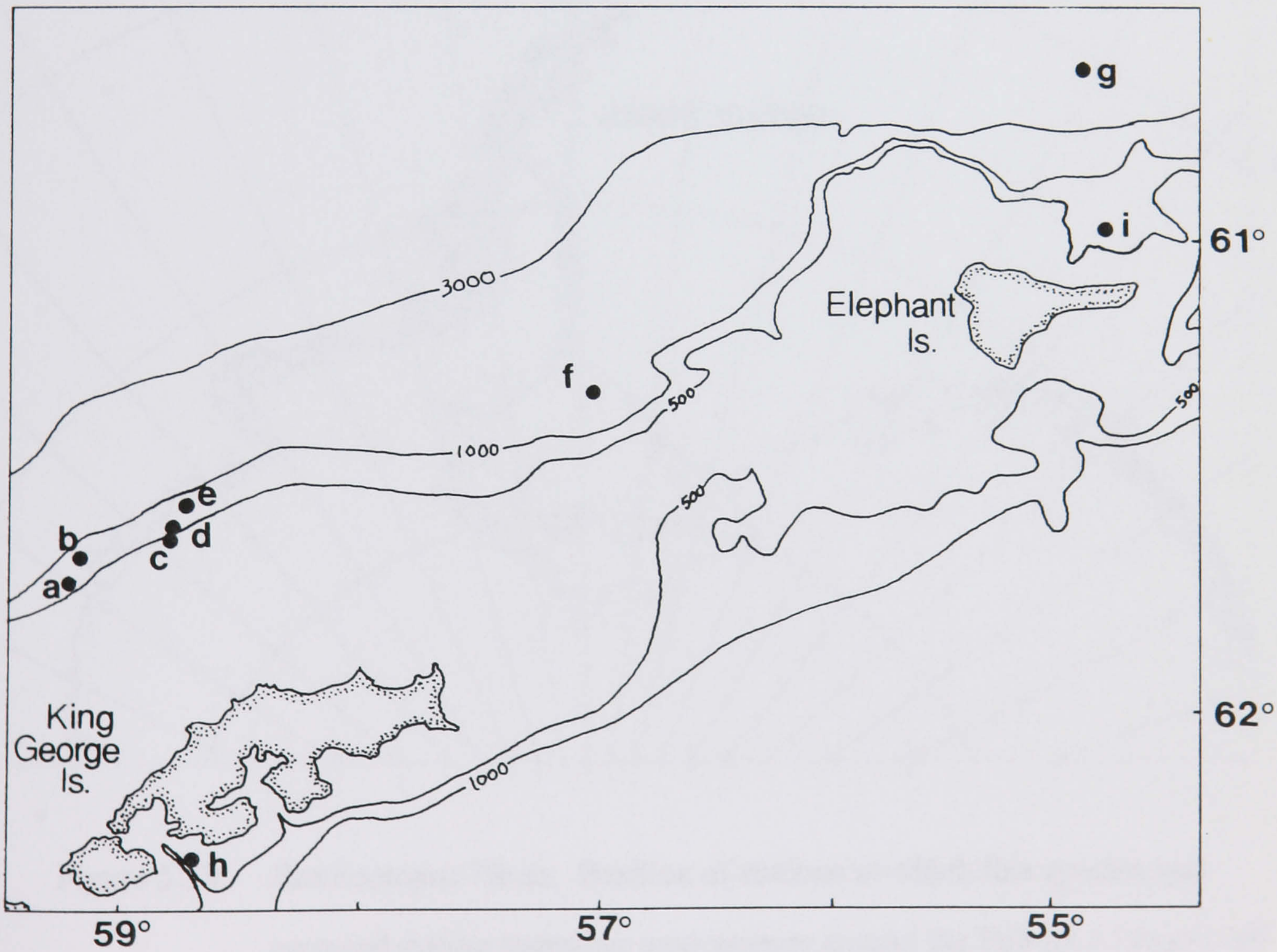


Figure 5.17: Capture locations of Graneledoninae (Voss, 1988a) during a benthic survey of the Antarctic Peninsula by *PFS Polarstern*. For details of species caught at each station refer to Table 5.3.

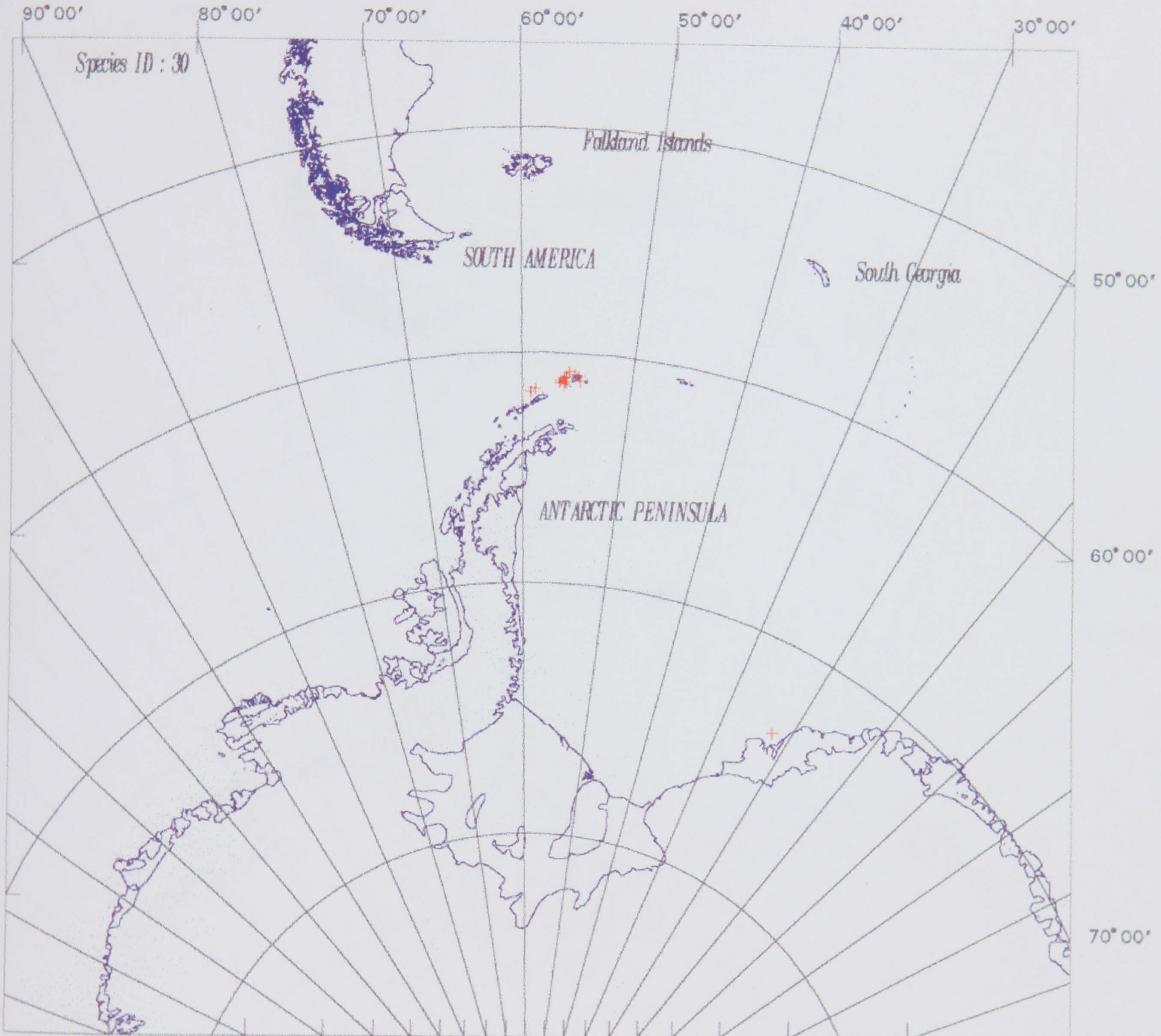


Figure 5.18: *Benthoctopus ?levis*. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

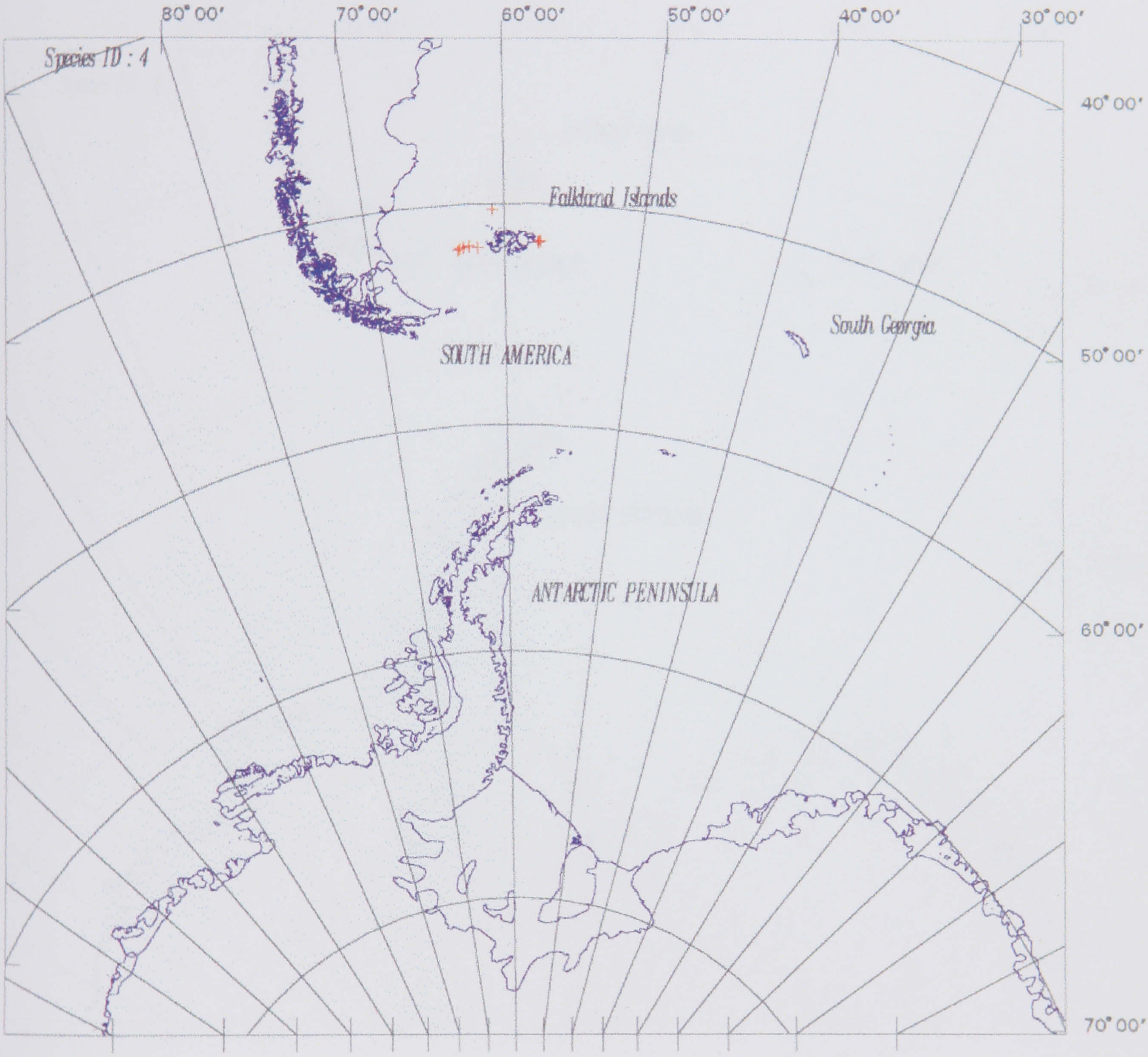


Figure 5.19: *Benthoctopus eureka*. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

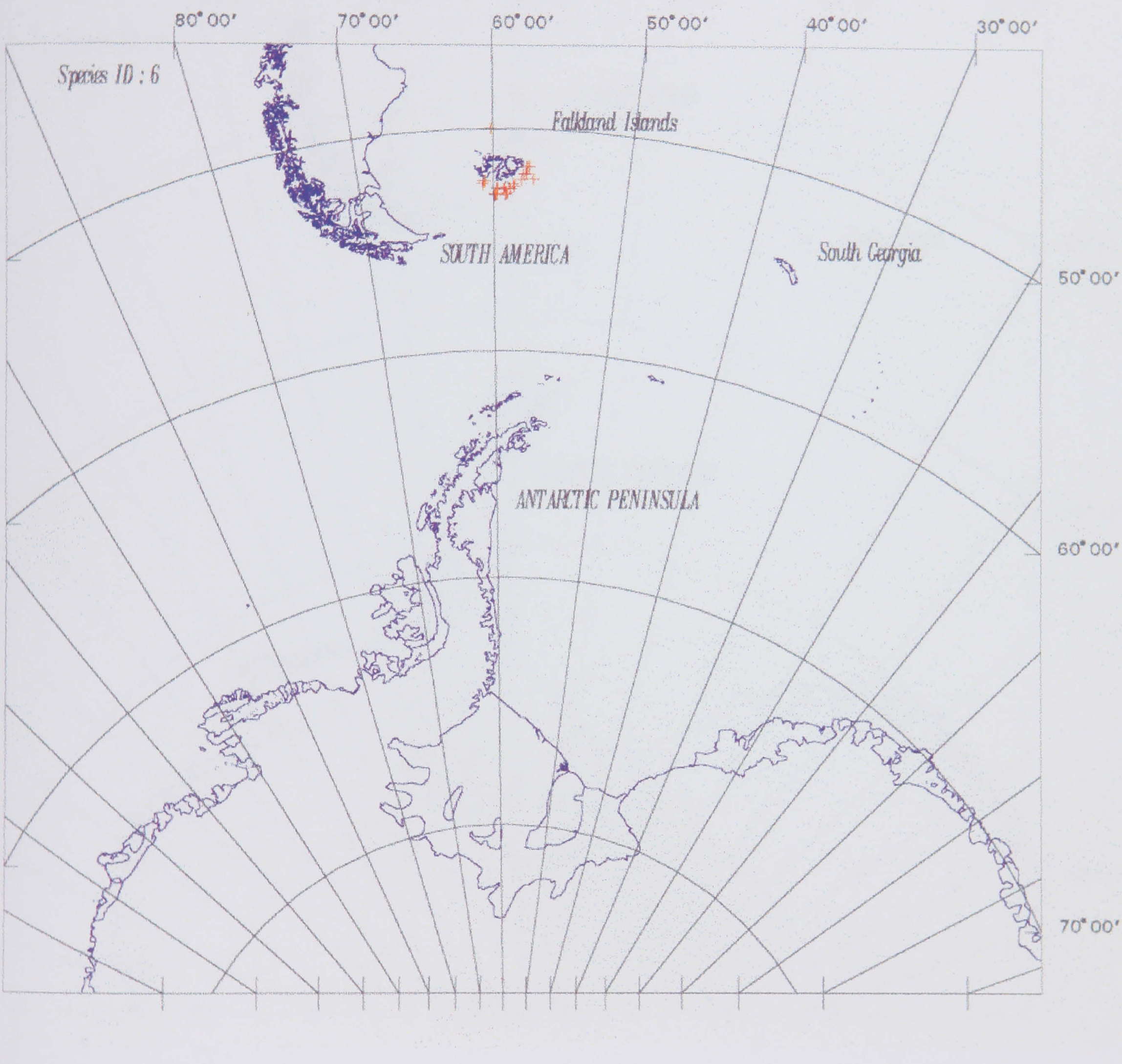


Figure 5.20: *Enteroctopus megalocyathus*. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

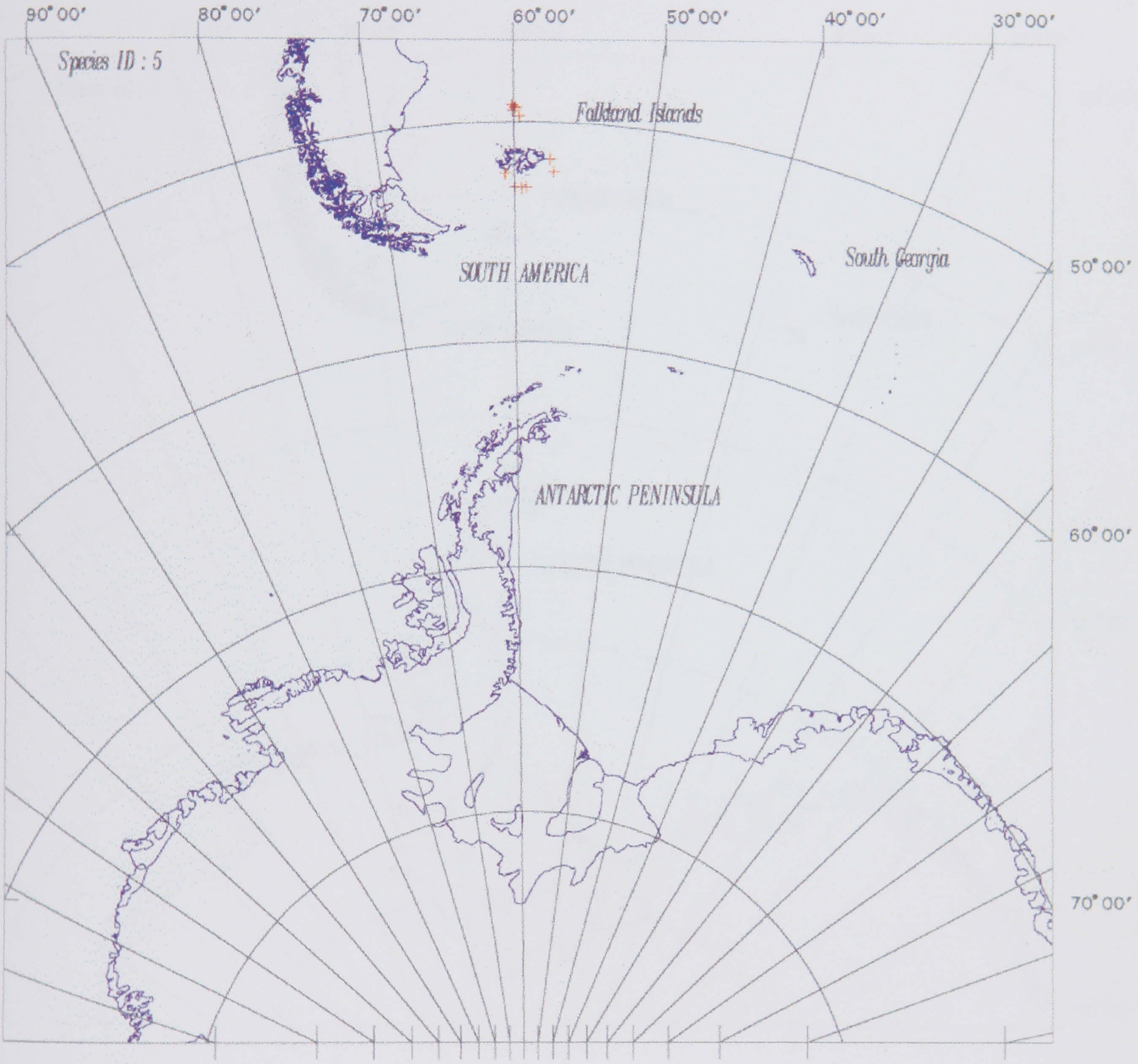


Figure 5.21: *Octopus tehuelchus*. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

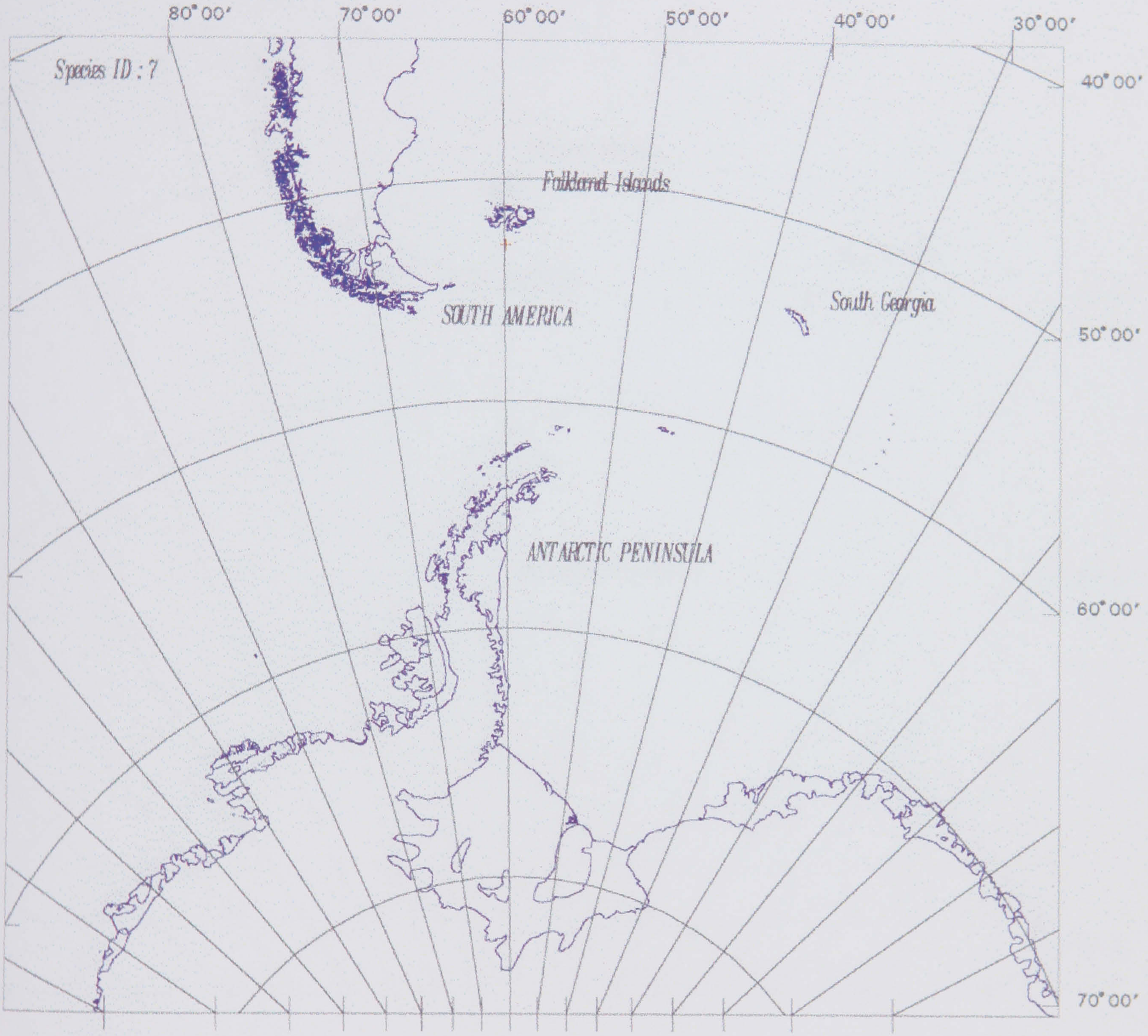


Figure 5.22: Sp. 7. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

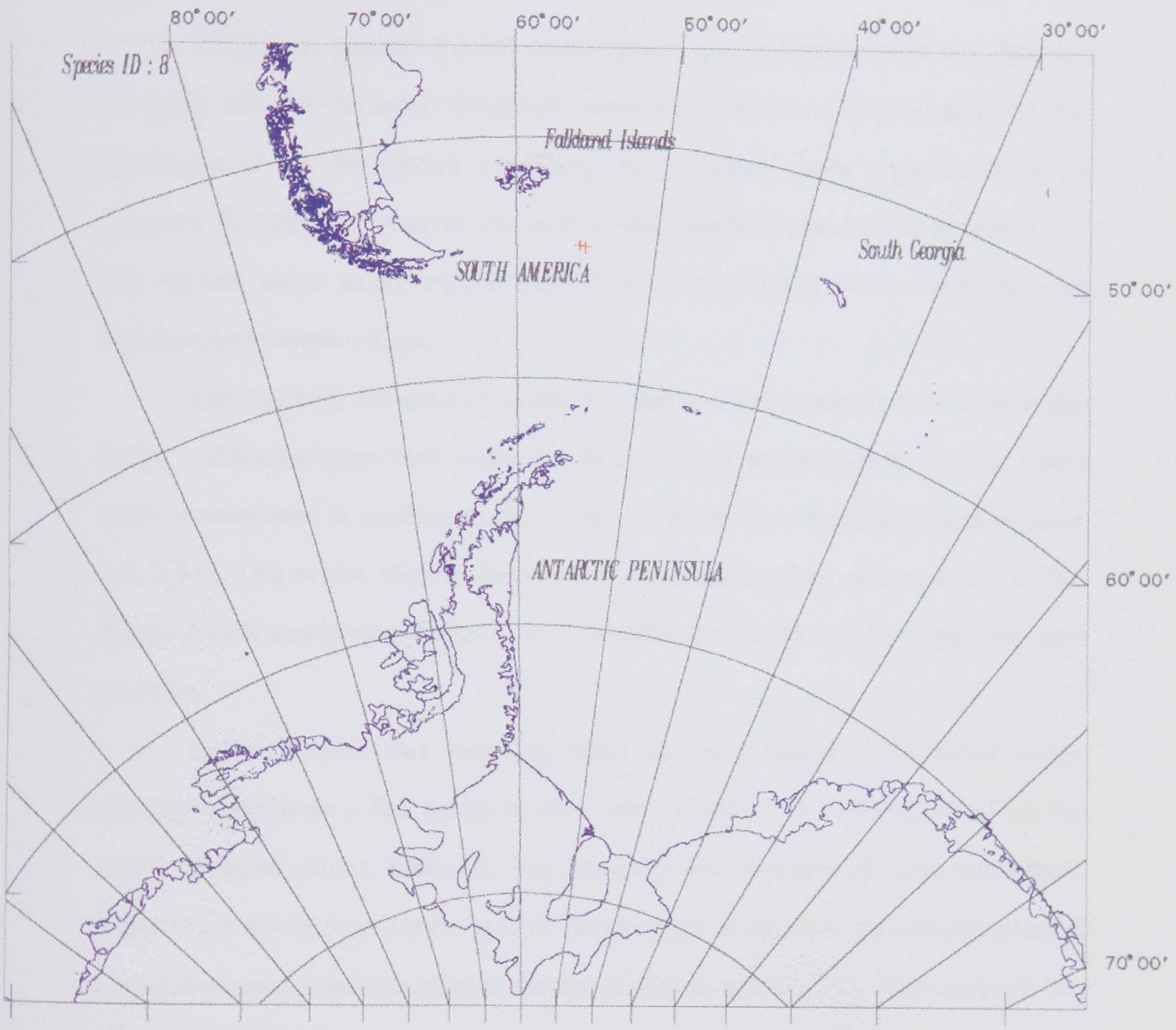


Figure 5.23: Sp. 8. Position of stations at which this species was captured during sampling programmes around the Falkland Islands, off South Georgia, off the Antarctic Peninsula and in the Weddell Sea.

outgroups and for comparisons. This collection of octopodids from the Falkland Islands is, however, extensive, and a series of maps illustrating the capture locations of each species is included (Figures 5.19-5.23) in the hope that it will be useful to future researchers.

Discussion

Whilst it is possible that all the octopodid species that occur in less than 600 m depth off the Antarctic Peninsula were captured, it is highly unlikely that specimens of all the species inhabiting deeper waters were represented in the samples. Indeed, there were no trawls at all between 800 m and 1,500 m depth, so if any species occur solely within these depth ranges, there would have been no opportunity to capture them.

The Antarctic Peninsula was the area that was fished most extensively in this study. Although there were many trawls conducted around South Georgia, these were concentrated at shallow depths (<300 m) where two species abound (Figures 5.3, 5.13). One of five slightly deeper trawls revealed another species, and if further deeper trawls had been undertaken it is possible that more species may have been revealed.

In the Weddell Sea sampling effort was also biased to shallower waters although there were a few trawls in deep water (Table 5.1). Fishing effort on this multi-discipline cruise, however, was generally low because of time constraints. There were not enough trawls at each depth range to produce meaningful plots of cumulative species number against number of trawls, and this fact alone suggests that the Weddell Sea has not been sampled adequately for octopodids. Again, this is especially true of the deeper waters.

It is possible that the octopus fauna of deeper waters will not, however, differ so greatly between regions as the shallow water fauna. The shallow water fauna are separated by tracts of deep water which subdivide the populations geographically and this may eventually result in speciation (see Chapters 6 and 7). Deep water fauna are

possibly more continuously distributed throughout the Southern Ocean. They may be able to maintain panmixia over much larger areas and allopatric speciation (see Chapter 7) would be much less likely to occur. Certainly, amongst the *Pareledone*, it appears to be the species that are restricted to a narrow depth range in reasonably shallow waters that have the most localised distributions. *P.* sp. 11 sp. nov. (354-571 m) and *P.* sp. 10 sp. nov. (212-481 m) are apparently both restricted to the Weddell Sea, and *P.* sp. 15 sp. nov. (268-512 m), *P.* sp. 16 sp. nov. (70-419 m) and *P.* sp. 19 sp. nov. (121-365 m) appear to be restricted to the area around the Antarctic Peninsula. *P. charcoti*, *P. turqueti* and *P.* sp. 13 sp. nov., whose depth ranges are somewhat larger (Figure 5.2), have a more cosmopolitan distribution (Figures 5.3, 5.4, 5.7). On this basis, it might be expected that *P.* sp. 14 sp. nov., which is restricted to deeper water but appears to have a reasonably wide depth range (Figure 5.2), would have been found at locations other than the Antarctic Peninsula. However, since fishing at the depths at which it occurs has been extremely limited in other locations, the fact that it has not been recorded at these locations does not preclude its existence.

The two species of *Pareledone* described by Lu and Stranks (1994), *P. framensis* and *P. prydzensis*, that have only been recorded from the type localities also appear to have narrow depth distributions, although it is possible that sampling was limited to certain depths.

Similar trends were seen amongst other members of the subfamily Eledoninae (Figure 5.2) with "*Pareledone*" *polymorpha* and *Megaleledone senoi*, both of whom have extensive depth ranges, having extended distributions (Figures 5.12, 5.13). It is also possible, however, that some species are adapted to particular habitats. "*Pareledone*" sp. 28 was found at only one station at 600 m depth off the Antarctic Peninsula. The depth was reasonably well fished (see above) but the haul in which "*P.*" sp. 28 was found consisted almost entirely of mud (Figure 5.2). Since nets usually contained an assortment of small rocks and stones, indicative of a hard

substrate, it is possible that this species was adapted for, and restricted in its distribution to, softer substrates.

Members of the subfamily Graneledoninae were found from 386 m depth to below 3,000 m depth. Voss (1988b) describes them as "deep dwellers, living between 1,000 m to in excess of 3,500 m", but their distribution certainly extends into shallower waters. The majority of intensive benthic surveys, however, are concentrated at even shallower depths, hence few data are available on the distribution of this subfamily either from this, or from previous studies. Previous capture locations of *Thaumeledone brevis* (Ross Sea), *Bentheledone albida* (Ross Sea) and *Graneledone antarctica* (Eastern Antarctica) (Voss, 1998b) suggest that these deep water species may have a cosmopolitan distribution.

The same is possibly true for the single species of the subfamily Bathypolypodinae encountered in this study, *Benthoctopus ?levis*. If this is confirmed as conspecific with Hoyle's (1885) specimen (Vecchione *et al.*, in prep), then the distribution of this species extends at least from Heard Island, to the Weddell Sea, to the Antarctic Peninsula.

Generally, however, the octopus fauna of the Southern Ocean appears to be particularly fragmented, with each new region that is surveyed yielding additional new species of octopodid. In Chapter 6 gene flow is investigated in one of the more cosmopolitan species, *Pareledone turqueti*, in an attempt to assess the geographic range over which species can maintain panmixia. In the light of these results, the apparent fragmentation of the Southern Ocean octopus fauna is then discussed in further depth in Chapter 7.

Chapter 6

Restricted gene flow across geographic barriers in *Pareledone turqueti*.

Introduction

Pareledone turqueti (Joubin, 1905) is extremely abundant around South Georgia and Shag rocks, islands of the Scotia Ridge, a sea floor rise in the Atlantic sector of the Southern Ocean (Figure 6.1). In this region *P. turqueti* provides a component of the diet of, for example, southern elephant seals, *Mirounga leonina*, (Rodhouse *et al.*, 1992), black-browed albatross, *Diomedea melanophris*, (e.g. Rodhouse, 1990) and blue-eyed shags, *Phalacrocorax atriceps*, (Wanless *et al.*, 1992) and is therefore of considerable ecological importance. Despite this *P. turqueti* has received little scientific attention until recently and hence knowledge of its biology is limited. Initial investigations have been morphologically based (Daly and Rodhouse, 1994; Daly, 1996), but, as yet, there has been no research into population dynamics - a field that has attracted increasing interest amongst cephalopod biologists in recent years (Brierley *et al.*, 1995; Boyle and von Boletzky, 1996).

In octopodids fertilisation takes place internally following a direct mating encounter and adult migration is often limited (e.g. Van Heukelem, 1973; Kayes, 1974; Ambrose, 1982; Mather *et al.*, 1985), so the geographic extent of genetic homogeneity within species should correlate with the degree of paralarval dispersal. As in many species of octopodid, however, the paralarvae of *Pareledone turqueti* have not been identified and the form they take is uncertain. Octopodid paralarvae may be either benthic or planktonic with paralarval type apparently related to the size of the egg capsule (Hochberg *et al.*, 1992). Eggs over 10 mm capsule length commonly produce demersal, crawl-away young whereas smaller eggs give rise to planktonic hatchlings. In *P. turqueti* egg capsules appear to reach a maximum length of approximately 18 mm (see Chapter 4) suggesting the hatchlings may be benthic in habit. It is unlikely that these crawl-away juveniles can survive in greater depths than the adults and the bathymetry of the Southern Ocean (where small islands are separated by great depths) will therefore provide many large barriers to dispersal.

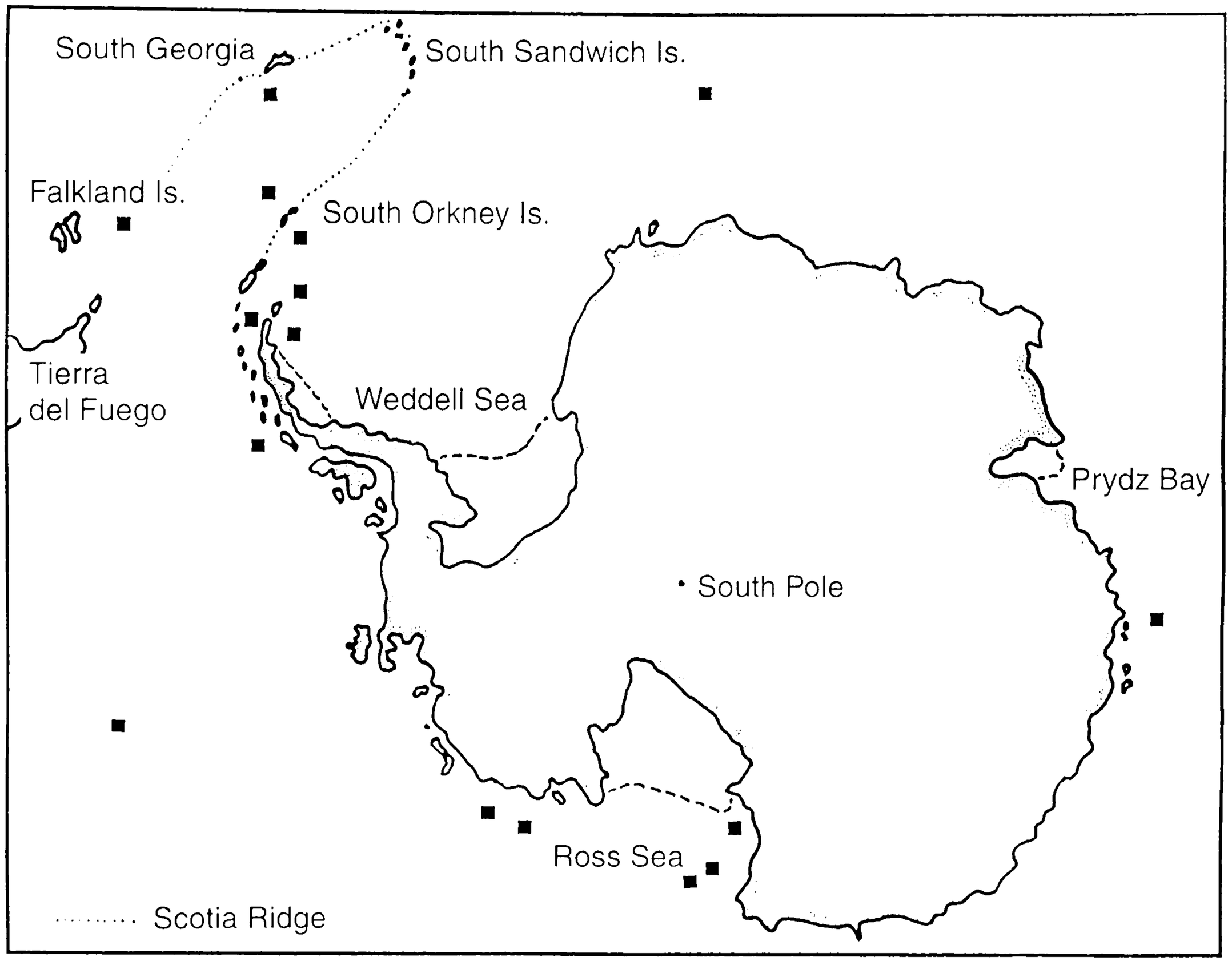


Figure 6.1: Map of Antarctic region showing previous capture locations of *Pareledone turqueti*. (Adapted from Voss (1988b), but see also Chapters 1 and 4.)

Although rarely applied to octopod biology, isozyme electrophoresis has revealed many cases of population heterogeneity in squid (Garthwaite *et al.*, 1989; Brierley *et al.*, 1993a) and highlighted the frequent occurrence of cryptic speciation in this group (Smith *et al.*, 1987; Carvalho *et al.*, 1992; Brierley *et al.*, 1993b). In the present study isozyme electrophoresis was applied to *Pareledone turqueti* specimens collected from Shag Rocks and from South Georgia, adjacent islands on the Scotia Ridge (Figure 6.1). These locations are separated by less than 150 kilometres of sea (Figure 6.2), of which approximately 30 kilometres are deeper than 1,000 m, reaching a maximum depth of approximately 1,750 m. This putative barrier is small relative to most of those encountered across the distribution of *Pareledone turqueti* and if there is marked heterogeneity across this barrier it is likely that throughout its distribution in the Southern Ocean *P. turqueti* will be extremely genetically heterogeneous.

Materials and Methods

Field sampling

The study area was surveyed by the Falkland Islands fishery patrol vessel *MV Cordella* over the period 6 January - 8 February 1994. During this time bottom trawls of 30 minutes duration were conducted at 82 random sites in three areas: Shag Rocks (SR), North West South Georgia (NWSG) and South East South Georgia (SESG) (Figure 6.2). The net used was an FP120 commercial trawl with a net mesh of 160 mm in the upper wings decreasing to 40 mm in the cod end liner. It was fished at depths varying from 107-440 m. The *Pareledone turqueti* in the catch were hand sorted at sea and tissue samples from the dorsal mantle were dissected and stored frozen at -20°C.

Electrophoresis

Horizontal starch gel electrophoresis was carried out using standard procedures, as described in Chapter 2.

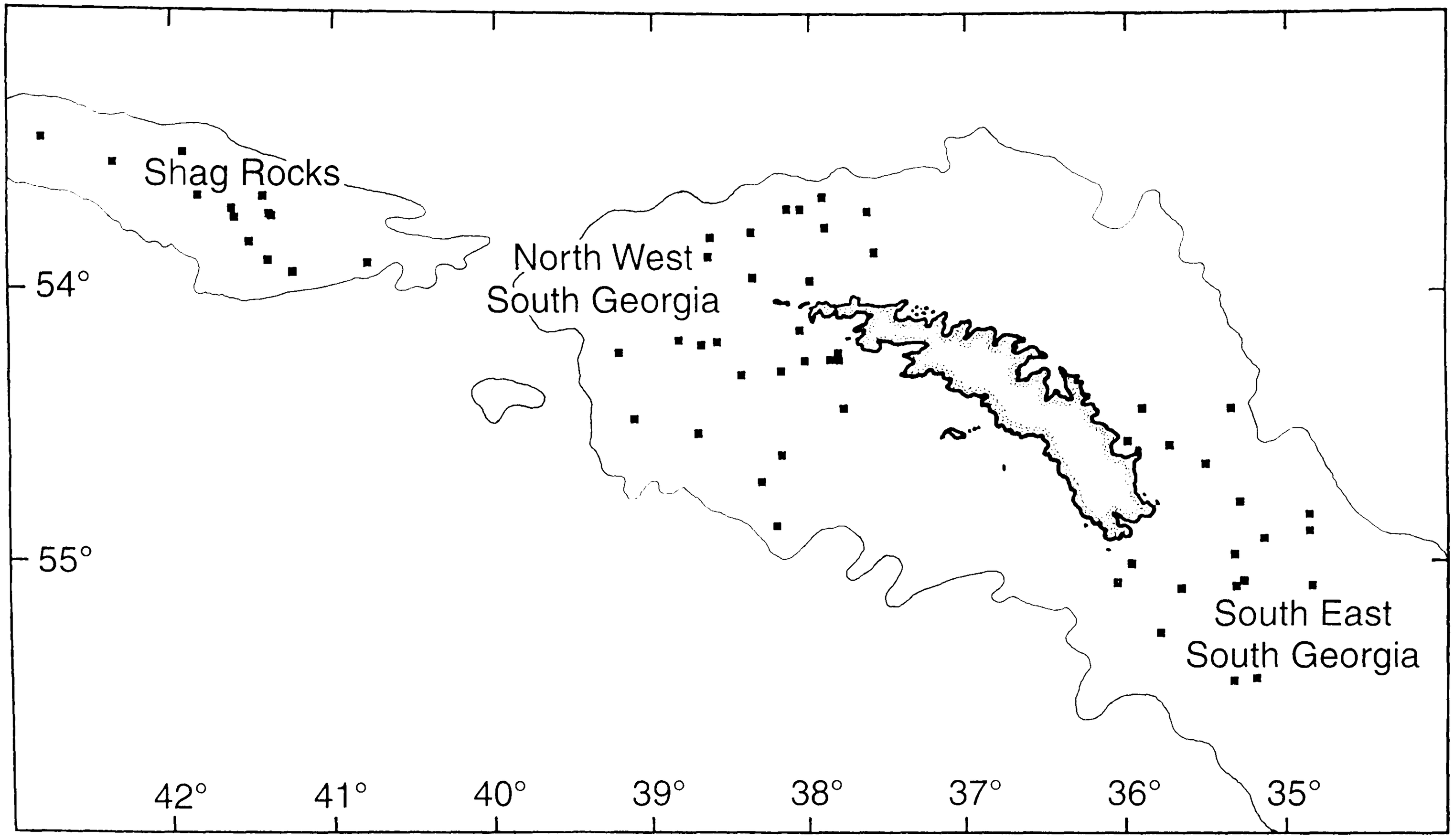


Figure 6.2: Sampling locations of *Pareledone turqueti* around Shag Rocks and South Georgia during 6 January - 8 February 1994. Thin black line represents 1000 m contour.

Data analysis

Genotypes and allele frequencies were analysed using the computer programs BIOSYS-1 (Release 1.7) (Swofford and Selander, 1981) and FSTAT (Release 1.2) (Goudet, 1994). The fixation index, *f*, (Weir and Cockerham 1984) was calculated for each population and tested for significant deviation from zero at the 95% confidence level using the normal approximation to the χ^2 distribution (i.e., if $f\sqrt{n} > 1.96$ then H_0 is rejected, where n is the number of individuals in the population sample and the null hypothesis is H_0: $f = 0$). An estimate of F_{ST}, θ, (Weir and Cockerham, 1984) was calculated across all populations. A pairwise matrix of θ was also constructed to indicate between which samples, if any, population subdivision occurred (Slatkin, 1993; Goudet, 1994). Genetic identity, *I*, (Nei, 1978) was calculated for pairwise comparisons of samples and clustered using UPGMA (unweighted pair group mean analysis) (Sneath and Sokal, 1973).

Results

Initially 54 enzymes were screened using a range of buffering regimes. This led to the resolution of ten enzymes on two buffer systems (Table 6.1). Two of the resolved loci were polymorphic and allele frequencies at these loci are given in Table 6.2. Mean observed heterozygosity per locus (H) was estimated as H = 0.024. The fixation index, *f*, was not found to differ significantly from zero in any of the samples (Table 6.3) and it was assumed therefore that each sample was drawn from a single breeding unit not deviating significantly from Hardy-Weinberg equilibrium.

The estimate of F_{ST}, θ, calculated across all populations was found to be 0.544. The pairwise matrix of θ suggests that the Shag Rocks sample is genetically distinct from those from South Georgia (Table 6.4).

Cluster analysis of Nei's (1978) genetic identity (Figure 6.3) supports the conclusions drawn from *F*-statistics.

Table 6.1: Enzymes resolved, most effective buffer system employed and number of loci detected. For buffer recipes see Murphy *et al.*, 1990. Stain recipes from Harris and Hopkinson, 1977 except †: recipe from Murphy *et al.*, 1990

Enzyme	EC No	Buffer	No of Loci
G3PDH	1.1.1.8	Tris-borate-EDTA II	1
PEP leu-tyr	3.4.13.*	Tris-borate-EDTA II	1
OPDH †	1.5.1.11	Tris-borate-EDTA II	1
SOD	1.15.1.1	Tris-borate-EDTA II	1
AAT	2.6.1.1	Tris-borate-EDTA II	1
ICD	1.1.1.42	Tris-citrate III	1
MDH	1.1.1.37	Tris-citrate III	2
MDHP	1.1.1.40	Tris-citrate III	1
MPI	5.3.1.8	Tris-citrate III	1
G6PDH	1.1.1.49	Tris-citrate III	1

Table 6.2: Allele frequencies at two polymorphic loci in three samples of *Pareledone turqueti.*

	Sample location		
Locus	Shag Rocks	N W South Georgia	S E South Georgia
G3pdh			
(n)	46	230	83
A	0.478	0.935	0.940
B	0.522	0.065	0.048
C	0.000	0.000	0.012
Icd			
(n)	46	230	83
A	0.185	0.954	0.946
B	0.815	0.046	0.054

Table 6.3: Tests for departure from Hardy-Weinberg equilibrium where the null hypothesis is Ho: $f = 0$. f, Weir and Cockerham's fixation index; n, number of individuals in the sample.

Sample	f	$f\sqrt{n}$	Reject Ho?
Shag Rocks	0.126	0.85	No
North West South Georgia	0.062	0.56	No
South East South Georgia	0.020	0.30	No

Table 6.4: Pairwise estimates of Weir and Cockerham's (1984) F-statistic, θ, amongst three samples of *Pareledone turqueti.*

	Shag Rocks	North West South Georgia
North West South Georgia	0.66	
South East South Georgia	0.72	0.00

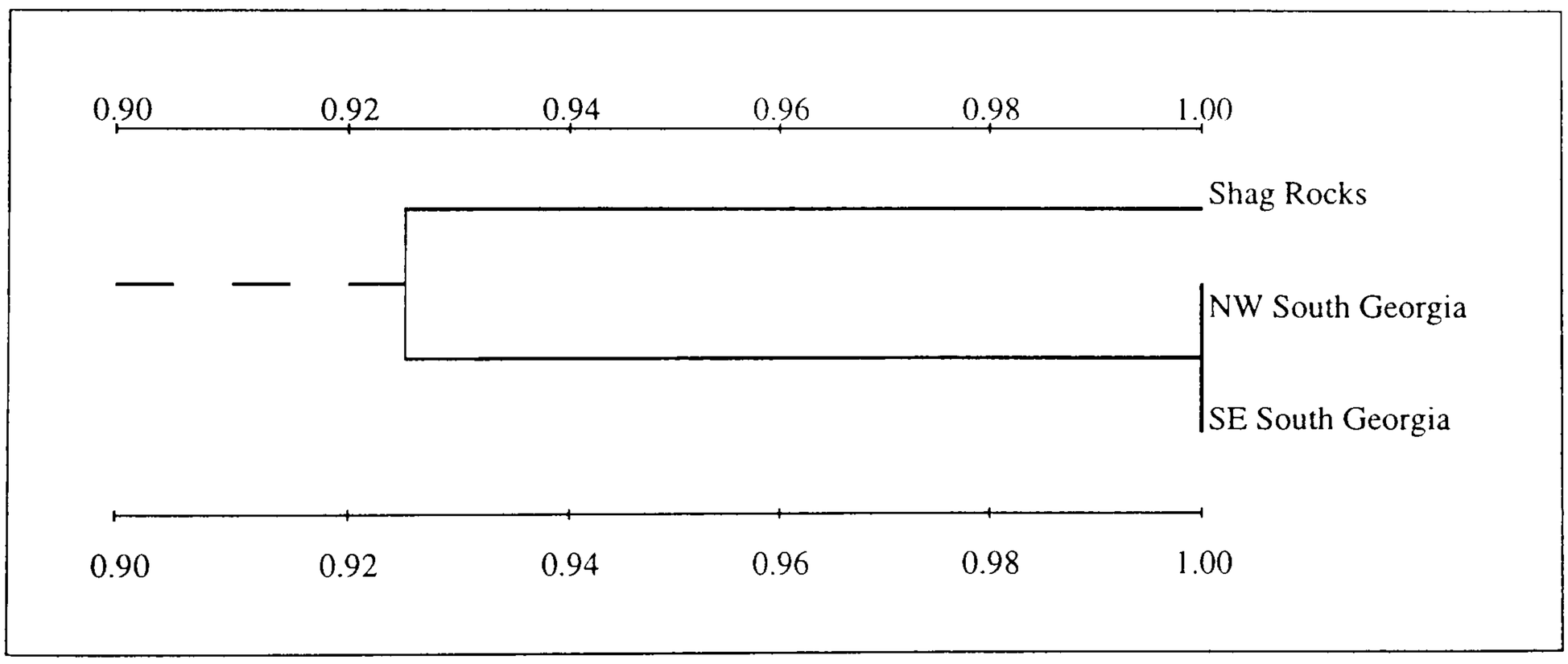


Figure 6.3: UPGMA dendrogram of Nei's (1978) genetic identity between three samples of *Pareledone turqueti.*

Discussion

Larval ecology strongly influences the structuring of a population and there has been much speculation concerning the evolutionary advantages of pelagic larvae (reviewed by e.g. Strathmann, 1985; Todd, 1985). If panmixia is maintained in geographically widespread marine species this is often due to the dispersal of planktonic larvae in ocean currents. In a species where the paralarvae are benthic and thus occupy the same physical niche as the adults, however, larval dispersal can be more limited, and the ability to cross large areas uninhabitable by the adult form may be lost. Many species of octopus, including *Octopus vulgaris*, that were once thought to be cosmopolitan now appear to be geographically fragmented (see Boyle and von Boletzky, 1996). Wright (1978) produced a classification scale for *F*-statistics. Values can range between 0 and 1 where 0 represents complete genetic homogeneity, 1 represents complete genetic heterogeneity (i.e., no alleles in common), and any value greater than 0.25 is indicative of "very great genetic differentiation" (Wright, 1978). The pairwise matrix of θ (Table 6.4) therefore suggests that populations around South Georgia are panmictic. Although an imbalance of sample sizes may have had a slight influence on the statistical analysis it is apparent that, in contrast, geneflow between South Georgia and Shag Rocks is severely restricted. The apparent genetic homogeneity between the two South Georgia samples suggests that *P. turqueti* is capable of maintaining panmixia in shallow on-shelf waters over a range of at least 150 km. Tracts of deep ocean (>1,000 m) however, as between South Georgia and Shag Rocks, apparently present a major physical barrier to dispersal and impose consequences for geneflow. The *F*-statistic value obtained between South Georgia and Shag Rocks suggests that there is almost no migration between the two populations in these areas. Of 31 examples of *F*-statistic values presented by Wright (1978) only one (that for the Caribbean lizard, *Anolis brevirostris*, at 0.78) exceeds the values calculated here (Table 6.4) and Larson *et al.*, (1984) suggest that F_{ST} values of this order are indicative of populations completely isolated from genetic exchange. This is remarkable when it is considered that the

two demes that were sampled are probably separated by not more than 30 km of deep ocean.

Although octopuses can swim fast for short periods of time their normal movement is a slow crawl and migration in this manner across unsuitable habitats is unlikely. In many marine organisms with non-planktonic larvae rafting has been suggested as an alternative dispersal mechanism (e.g. Johannesson, 1988; Jokiel, 1989; Parker and Tunnicliffe, 1994). This has been shown to occur on, for example, both floating and submerged drift algae (Worcester, 1994 c.f. Holmquist, 1994) and is not confined to species normally inhabiting algae (Ingolfsson, 1995). Furthermore, it has been shown that kelp-rafting may serve as a 'significant means of genetic exchange between populations' in a sub-antarctic brooding mollusc (Helmuth *et al.*, 1994). Since the *F*-statistic values indicate that there is little genetic exchange between South Georgia and Shag Rocks neither rafting nor any other mechanism seems to be acting to facilitate geneflow between these areas. Evidence from studies on fish also suggests that there is a barrier to planktotrophic dispersal in this region. Temporal differences in maturation as well as genetic differences have been found in Antarctic icefish, *Champsocephalus gunnari*, from Shag Rocks and South Georgia (G. Carvalho, pers. comm., 1995), and differences have also been found in the infestation loads of digenean trematodes in other species of inshore fish from the two sites (Zdzitowiecki and White, 1992). Furthermore another fish species, *Patagonotothen guntheri*, which is found in depths of 120-250 m and which has a geographical range from Patagonia to Shag Rocks, has never been reported from South Georgia although data from commercial catches indicate that it spawns in the Shag Rocks area (Dewitt *et al.*, 1990).

Given the degree of genetic separation between South Georgia and Shag Rocks, it is likely that the *Pareledone turqueti* of the Scotia Ridge (Figure 6.1) will comprise several isolated populations. Although in the Scotia Ridge island chain distances between populations may be as little as a few kilometres (e.g. for islands of the South Sandwich group) they range up to 500 kilometres (e.g. between the South

Sandwich Islands and the South Orkney Islands). The island populations of *P. turqueti* are apparently surrounded by insurmountable barriers to geneflow and each is likely to exhibit the genetic manifestations of isolation, which over time may lead to speciation.

Along the coast of continental Antarctica there are no obvious barriers to geneflow and there geographical distance between sites is likely to constitute the major restriction on geneflow. Wright (1943) described a situation where the migration distance of an individual was very small in comparison to the area over which the species was continuously distributed. This prevented the species from forming a single panmictic unit and produces 'isolation by distance'. Data presented here indicate that *Pareledone turqueti* can maintain panmixia over 150 km (between NWSG and SESG). It is not known, however, whether *P. turqueti* is distributed as densely around continental Antarctica, as sampling in other areas was with a variety of gears and catches were not, therefore, comparable quantitatively. Even if the density of *P. turqueti* were fairly high across its distribution, it is unlikely that any species without a planktonic larval stage could maintain panmixia over a linear distribution covering thousands of kilometres of coastline.

Although there have been reports of *Pareledone turqueti* from all around Antarctica (Figure 6.1) doubt has been cast on some of the identifications involved (see Chapters 1 and 4). In the light of the heterogeneity shown over such a small distance, it is highly unlikely that specimens reported from the Ross Sea and near to Prydz Bay are in fact *P. turqueti.* These data support the assumption made in Chapter 4 that the distribution of *P. turqueti* does not extend beyond Western Antarctica.

Ideally it would have been preferable to study allele frequencies at polymorphic loci in samples from the Weddell Sea and from the Antarctic Peninsula. Unfortunately, the enzyme ICD did not appear to be polymorphic in samples from these locations, and, because of enzyme degradation, G3PDH could not be scored in samples from the Antarctic Peninsula (Chapter 2, Table 2.4). Although no

polymorphic loci were scored from the Antarctic Peninsula, the Weddell Sea samples could theoretically have been included on the basis of one locus. Estimates of gene flow based on a single locus are, however, unsatisfactory.

The lack of variation in octopuses (this study), cuttlefish (Sanjuan *et al.*, 1996) and squids (Ally and Keck, 1978; Thorpe *et al.*, 1986; Brierley *et al.*, 1993b), as detected by allozyme electrophoresis, decreases the usefulness of this technique in population genetics. Brierley (1992) discusses some of the theories that have been put forward to explain varying levels of polymorphism. These include theories from both the neutralist and selectionist viewpoints.

The neutralist viewpoint is that genetic variability is determined by effective population size and the intrinsic mutation rate (Kimura and Ohta, 1971; Johnson, 1973; Nei *et al.*, 1976; Kimura, 1983). Since the latter is considered to be fairly constant (Ward, 1977, 1978; Nei *et al.*, 1978; Thorpe, 1989), effective population size should drive the level of variation within a population.

In a review of data available in 1973, however, Selander and Kaufman discounted this hypothesis (albeit on the basis of preliminary evidence from *Drosophila* species). Instead they attempted to explain levels of variability in terms of the environmental grain theory (Levins and MacArthur, 1966; Levins, 1968). Levins proposed that the most important factors driving variability are individual motility and homeostatic control. Although the theory is based on how an organism 'perceives' its environment, the conclusions are simple; large mobile species should exhibit lower levels of genetic variability compared with small immobile species. The variability of many species fits this hypothesis (low levels of genetic variation in pelagic elasmobranchs cf. high levels of genetic variation in territorial demersal sharks (Smith, 1986); high levels of genetic variation in sessile coelenterates and sponges (Solé-Cava and Thorpe, 1989, 1991)), and Brierley (1992) argued that the variability of the Mollusca also fits the hypothesis. Certainly pelagic squids exhibit much lower levels of variability than do other more sessile molluscs (e.g., Garthwaite *et al.*, 1989; Carvalho and Loney, 1989 cf. Beaumont and Beveridge, 1984).

Antarctic octopuses, however, are certainly small and it is likely that their movement is limited, hence they should, under the environmental grain hypothesis, exhibit high levels of variability. Clearly, in this case, an alternative hypothesis is required.

The time divergence hypothesis (e.g., Soulé, 1972) predicts genetic variation will be greatest in populations that have existed for long time periods. The suggestion by Voss (1977) that ommastrephids, loliginids and octopods are currently undergoing speciation as a result of adaptive radiation fits with this hypothesis, as species within these groups must, by definition, be young in evolutionary terms and should therefore exhibit low levels of variability.

However, the environmental grain and time divergence hypothesis are only two of a huge variety of selectionist hypotheses put forward to explain genetic variability, and with so many theories available, it is usually possible to fit more than one to a small group such as the cephalopods, and this results in several conflicting explanations. The neutralist viewpoint (Kimura, 1983), based on effective population size, may also explain the data. One of the factors that may depress effective population size (and hence variability) is fluctuations in population size. The effective population size due to such fluctuations is equal to the harmonic mean of the breeding population sizes across generations (Avise, 1994). A harmonic mean is closer to the lower end of a series of numbers being analysed and consequently large fluctuations in actual population size can severely depress effective populations. Since *Pareledone* are thought to be semelparous (Daly, 1996), large fluctuations in their populations sizes are likely.

Some studies also indicate that endemic species have lower levels of variation than similar, more widespread species (Kruckeberg and Rabinowitz, 1985) although the results of other studies are contradictory to this (e.g., Ledig and Cronkle, 1983) and more research is required to validate such generalisations. It is unlikely, however, that this is the most suitable explanation for reduced variability in the *Pareledone* because, although this genus is endemic to the Antarctic, low variability is seen in many other non-endemic cephalopod species.

Whatever the mechanism driving the reduced variability in cephalopods, allozymes are probably not the best way to investigate population genetics in *Pareledone*, even though they have the advantage that heterozygotes can be detected. Molecular techniques, whilst providing greater sensitivity, have the advantage that DNA is generally more stable than proteins, and some of the problems encountered with degradation in this study might be avoided. There are many alternative molecular techniques, but there are few published data available for cephalopods. Several research groups are currently developing molecular techniques for cephalopod biology (e.g., Bonnaud *et al.*, 1994, 1996, 1997; Nishiguchi *et al.*, 1995; Boucher-Rodoni and Bonnaud, 1996), but these studies have mainly been aimed at higher level systematics. RAPDs have been promoted strongly by some authors (e.g., Hadrys *et al.*, 1992; Hedrick, 1992) as suitable for populations genetics but much discussion still surrounds the interpretation of the data they generate (e.g., Clark and Lanigan, 1993; Lynch and Milligan, 1994). Because of this, RAPDs may prove to be more suitable for limited applications such as identifying juveniles with their adult form (Allcock *et al.*, in prep). An attempt was made to sequence the COIII gene in the veined squid, *Loligo forbesi* (Norman *et al.*, 1994), and microsatellie markers have been developed for the same species (Shaw, 1997). It is likely that progress in the field of cephalopod population genetics will be in one of these directions.

Chapter 7

Biodiversity, speciation and cephalopod phylogeny: a discussion of factors pertinent to the radiation of the genus *Pareledone* in the Southern Ocean.

Most taxonomists would agree that the discovery, in two sampling seasons, of seven new species of a single genus amongst large and obvious creatures such as octopuses would be extremely unlikely. With this in mind, this chapter explores some of the wider aspects of taxonomy, such as biodiversity, speciation and phylogeny. By examining research on either other molluscan, or other Southern Ocean, species an attempt is made to outline some of the mechanisms that may have played a role in the radiation of the genus *Pareledone*.

When all five kingdoms are taken into consideration, it has been suggested that there are at least ten times as many extant species as we have discovered. These estimates come from experiments such as those that fog the rain forest canopy with insecticide, so as to comprehensively sample a limited area. However, balancing the discovery of obscure arthropods is the continuing discovery of new phyla (Morris, 1995), subfamilies (Marshall, 1996) and even large vertebrate species (Olson and Jouventin, 1996). In some groups e.g., the snail fishes (Liparididae), almost half the estimated 60 species are undescribed (Stein *et al.*, 1991).

It has generally been supposed that marine biodiversity decreases with depth, with some shallow water marine invertebrate communities exhibiting extremely high levels of diversity. If species totals are accurate for each ecosystem then the most speciose marine ecosystems, such as coral reefs, may have diversity levels similar to those seen in the most speciose terrestrial ecosystems, such as tropical rain forests (Hawksworth and Kalin-Arroyo, 1995). Recent studies of deep sea habitats in the North Atlantic (Grassle, 1991) and off Southwest Australia (Poore, 1993), however, have uncovered a level of species richness and diversity, unimagined in such environments, that rivals the levels seen on coral reefs. If this level of diversity is typical of more marine habitats then it is possible that the overwhelming number of terrestrial species (there are seven times more named terrestrial species than there are marine species; Lasserre, 1992; May, 1994) may simply reflect sampling bias. Certainly at the level of phyla, terrestrial diversity is only half that of marine

environments. Of 34 extant phyla (Margulis and Schwartz, 1988; Morris, 1995), 33 occur in the sea and sixteen are exclusively marine.

Although attempts have been made to explain large scale patterns of diversity the results are often conflicting. Both marine molluscs (Stehli *et al.*, 1967) and deep-water benthic invertebrates (Rex *et al.*, 1993) have been shown to increase in diversity towards the tropics, although separate studies on deep-water benthic invertebrates have concluded conversely that species diversity is consistently high at all latitudes (Grassle, 1989, 1991; Grassle and Maciolek, 1992). One of the most thorough studies, which examined plankton counts in five degree squares throughout the world's oceans (McGowan and Walker, 1993), illustrates that there is no simple trend, at least in plankton.

Of all the animal phyla only the Arthropoda, with 1,085,000 species (Hammond, 1992), is more speciose than the Mollusca. Figures for the number of molluscan species range from 45,000 to 150,000 (Minelli, 1993), with 75,000 estimated as the figure for the marine species of molluscs alone (Winston, 1992). The large number of molluscan species is reflected in Aristotle's early classification of invertebrates. This included five groups: the cephalopods; molluscs other than cephalopods (although this also included the echinoderms); crustaceans; other arthropods; sponges and coelenterates.

Cephalopods probably evolved from monoplacophorans during the late Cambrian (Yochelson *et al.*, 1973; Runnegar and Pojeta, 1974; Pojeta and Runnegar, 1976); Engeser (1990) suggests that the fossil monoplacophoran *Knightoconus* should actually be placed in the taxon Cephalopoda as a stem-lineage representative. Cephalopods underwent significant diversification in the early and middle Ordovician at a time when other Mollusca (e.g., monoplacophorans, archeogastropods, and at least two lineages of bivalves) were also diversifying (Runnegar and Pojeta, 1985). The first coleoids are found from the Devonian (Engeser, 1990) in the form of belemnites. The next fundamental change in the cephalopod fauna took place at the Cretaceous-Cenozoic boundary (Figure 7.1). The

Era	Period or Epoch		Ma
Cenozoic	Quaternary	Holocene	0
		Pleistocene	0.01
	Tertiary	Pliocene	2
		Miocene	5
		Oligocene	25
		Eocene	38
		Paleocene	55
Mesozoic	Cretaceous		65
	Jurassic		144
	Triassic		213
Paleozoic	Permian		248
	Carboniferous		286
	Devonian		360
	Silurian		408
	Ordovician		438
	Cambrian		505
Pre-Cambrian			590

Figure 7.1: Classification of geological time.
Ma, millions of years.

major extinctions of the late Cretaceous Period often detract from the massive diversification in marine fauna at the end of the Mesozoic Era. Although ammonoids and belemnites suffered during the mass extinctions of this period, the 'marine Mesozoic revolution' (Vermeij, 1977) led to the diversification of the coleoid cephalopods (sepiods, teuthoids and octopods) as well as the few nautiloids that survived the preceding extinction. The oldest true known octopod, *Palaeoctopus newboldi* (Woodward) dates from this period (Engeser, 1988). The late Cretaceous extinction was the last of the five major mass extinctions in the marine realm, and those cephalopods that survived the event have gradually evolved to produce the forms seen today.

During the Mesozoic revolution several important groups of predatory teleost fish (Taylor, 1981), shell breaking predators (Vermeij, 1987) and disruptive sediment movers (Thayer, 1983) also underwent rapid evolutionary expansion. The evolution of all these groups would have had a marked effect on the benthic fauna (a stable bottom environment had persisted throughout the Palaeozoic Era) and hence upon the adaptation and evolution of new species. Vermeij (1978) suggests that many features of gastropod shell ornamentation arose at this time as a defence to predation.

Whilst there is little doubt that predation pressures can drive evolution there are many other factors involved. Ehrlich and Raven's (1964) classic paper on the co-evolution of butterflies and plants has led to many other suggestions of evolutionary interactions in animals and plants and co-evolution is now thought to be a common result of host-parasite interactions. Co-speciation, where both new hosts and new associate species evolve together, is a special case of co-evolution, which may provide "a significant source of novel biodiversity" (Barbault and Sastrapradja, 1995). There is only one corroborative study for this theory. Phylogenies, based on enzymatic polymorphisms and mitochondrial genome sequences, of 15 species of rodent and 17 species of associated chewing lice, indicate that co-speciation is common within this group (Hafner and Nadler, 1988; Hafner *et al.*, 1994), at least.

Cephalopods are not without their parasitic counterparts - the dicyemid mesozoans. The traditional view is that digeneans (parasitic on other molluscan species) evolved from free-living turbellarians. It has been suggested however, that digeneans evolved from a common stock that also gave rise to the dicyemids, which in turn suggests that there has been an association between these parasites and their molluscan hosts since before the time when cephalopods separated from the ancestral molluscan stock (Wright and Southgate, 1981). A corollary of this is that cephalopod evolution may have been partially driven by the evolution of dicyemids since the late Cambrian Period, 500 million years ago. Although recent molecular work may suggest an alternative origin for the dicyemids (Katayama *et al.*, 1995) there is little doubt that this association has been a long one. With this in mind, researchers are currently examining the phylogenies of both dicemyids and squids in parallel (Nishiguchi *et al.*, 1995).

It has also been suggested that climatic stability may have a role to play in evolution. Cook (1991) uses the diverse snail fauna of Madeira (an island considerably smaller than Britain, but with a far more stable climate and twice as many snails) to illustrate the possible effects of climatic stability, and quotes Wallace (1878): "The equatorial zone, in short, exhibits to us the result of a comparatively continuous and unchecked development of organic forms; while in the temperate regions, there have been a series of periodical checks and extinctions of a more or less disastrous nature necessitating the commencement of the work of development in certain lines over and over again. In the one, evolution has had a fair chance; in the other it has had countless difficulties thrown in its way".

Work on predatory gastropods (Taylor *et al.*, 1980) suggests that the tropics may not always have been characterised by high levels of diversity, however, and such levels may in fact be explained by long term instability. Glacial advances and regressions during the Pleistocene may have led to both wet and dry phases in the tropics, and hence the formation of fragmented habitats, which in turn may lead to allopatric speciation (see below). In reality, instability probably leads to speciation,

but also to extinction, and greatest diversity occurs in regions which are climatically stable over years or hundreds of years, but are geologically or climatically unstable on a time scale of 10^5-10^6 years (Cook, 1991). The climate of the Southern Ocean is undoubtedly unstable over larger time periods, as the effect of glacial expansion would be to prevent primary production in areas that currently enjoy a seasonal phytoplankton bloom. However, the climate should probably also be considered unstable over shorter time periods; Antarctic seasons are certainly extreme when compared to those experienced in the tropics.

For many years evolutionary rate was considered to be stable with respect to sidereal time. If this assumption is correct, the *Pareledone* should have been evolving steadily for many years, and it is surprising that some of the species are morphologically so similar (e.g., the smooth skinned *Pareledone*: *P. turqueti, P. harrissoni, P.* sp. 14 sp. nov. and *P.* sp. 13 sp. nov.) when they are clearly differentiated genetically. In 1972, however, Eldredge and Gould published the theory of punctuated equilibria, which proposes a general stasis for organismal lineages except during speciation events. The theory refers primarily to patterns of morphological divergence (Avise, 1994), as characters such as allozymes and DNA sequences should act as neutral molecular characters (Kimura, 1983). Williamson (1981) provided evidence to support this theory from the fossil record. He studied freshwater molluscs in African lake deposits. His specimens covered 4 million years and contained 19 species lineages in 18 genera of gastropods and bivalves and showed that long periods of stasis had been punctuated by rapid (5,000-50,000 years) changes in shell shape.

Neontological evidence for periods of morphological stasis is provided by Larson (1984) (although other studies refute the theory; e.g., Lemen and Freeman, 1989) in a study of plethodontid salamanders. He identified a minimum of 15 speciation events, only three of which could have been accompanied by substantial morphological change. Biochemical genetic studies indicated that genetic isolation

between populations of salamanders is ancient, and hence that there is strong morphological evolutionary stasis in this group.

The most common explanation for morphological evolutionary stasis is 'stabilising selection' (Charlesworth *et al.*, 1982; Williamson, 1987) whereby individuals that deviate from a standard phenotype are eliminated. Williamson proposes that the developmental stability of novel phenotypes prevents their persistence when in competition with established phenotypes. This would be especially true at periods of high population density and novel forms would appear when selection pressures were lower, for example during a rapid population expansion following a severe founder event (Carson and Templeton, 1984). Hence morphological change may be a delayed consequence of speciation. It is possible that the current abundance of octopodids in Antarctic waters has persisted for a considerable amount of time. The high selection pressures resulting from this abundance may have depressed the development of novel morphological forms, although evolution at the molecular level (Kimura, 1983) would have continued at a constant rate. This would be a plausible explanation for the morphological similarity of a number of *Pareledone* species, which exhibit clear divergence at the molecular level.

According to current systematic schemes, there have been two major evolutionary events within the Octopodidae (Voss, 1988a). The first is the change from a uniserial sucker arrangement to a biserial arrangement. Voight (1993c) studied the number and arrangement of suckers across a range of species and concluded that "if the number of suckers exceeds a critical limit dependent on arm length, the suckers form double rows" and hence "that the number of sucker rows is in fact a continuous character". Although Voight feels that the number of sucker rows should not be used as a dichotomous character, genetic evidence presented here (Chapter 2) suggests that there is genetic separation between animals with uniserial suckers and those with biserial suckers. When a dendrogram of Nei's *I* was constructed based on eight enzyme loci, each group formed a distinct cluster and

these were clearly divergent (Figure 2.3); the only exception to this was that *Eledone cirrhosa* appeared more closely related to species with biserial suckers, although it was not particularly closely related to either group. *E. cirrhosa* has long been considered an anomaly with respect to sucker arrangement (see Chapter 1; Robson, 1932; Voss, 1988a) and recent studies indicate that *E. cirrhosa* is not a true *Eledone* and should be moved to a new genus (F. G. Hochberg, pers. comm., 1997). When this is taken into consideration, evidence presented in this thesis (Chapter 2) supports the view that sucker serialisation is a dichotomous character that reflects evolutionary relationships.

The second major event in octopus evolutionary history was the loss of the ink sac. This has apparently happened twice (Voss, 1988a), once in animals with uniserial suckers and once in those with biserial suckers. Absence of the ink sac has usually been considered a convergent character (Robson, 1925, 1932; Voss, 1967, 1988a, b), yet there is little consideration given to the argument that if such a loss can occur twice, it might easily occur on numerous occasions. Robson (1932) states "It is uncertain whether the absence of the sac in the Cirromorpha is primitive or an adaptation to abyssal conditions. The absence of the sac in the Bathypolypodinae is probably a specialisation." Presumably by this he was suggesting that the loss of the ink sac in the Bathypolypodinae may also be an adaptation to depth. With light intensity varying inversely with depth, the expression of a cloud of ink to confuse visual predators will obviously decline in effectiveness with depth. Selection pressure for this character will therefore be reduced at depth: there may even by selection pressure against this character if it is energetically more efficient not to produce ink. If loss of the ink sac is an advantageous adaptation in abyssal creatures then presumably there is nothing to prevent this adaptation occurring again and again. Until now, the presence or absence of the ink sac has been included in the diagnosis of a genus. However, the discovery of "*Pareledone*" sp. 18 (See Chapter 4), in which the ink sac is either considerably reduced or apparently absent confuses an issue that has previously been clear cut, as this species is very definitely a sister species of

"Pareledone" polymorpha which consistently has a well defined ink sac. In Chapter 2, a single *Pareledone* specimen (*P.* sp. 27), which had been thought to differ from *P.* sp. 14 sp. nov. on the basis of external colour patterns, was found to be genetically identical at eight enzyme loci to *P.* sp. 14 sp. nov.. The ink sac of this specimen appeared to be reduced or possibly absent, but this feature was overlooked as an anomaly at the time as the specimen was clearly *Pareledone*, and hence, under current taxonomic thinking, must have an ink sac; the specimen was considered conspecific with *P.* sp. 14 sp. nov., although in retrospect it probably needs re-examining. Both "*Pareledone*" sp. 18 (570-1,523 m) and *Pareledone* sp. 27 (1,523 m) were trawled from considerable depths and there is mounting evidence that loss of the ink sac is an adaptation to depth which has occurred many times. The dendrogram of Nei's *I* constucted with data from eight enzyme loci (Chapter 2, Figure 2.3) showed no clear divergence between uniserial suckered species with ink sacs and uniserial suckered species without ink sacs. Whilst the conclusions drawn here may be contentious, they have obvious implications for octopodid taxonomy schemes.

With even the supposed major evolutionary events being debated by various authors it is unsurprising that there is so much confusion surrounding octopodid taxonomy. Voight (1993a) suggests that cladistics is the way forward but in her cladistic reassessment of octopodid phylogeny (Voight, 1993b) acknowledges that the rationale behind the polarity definitions for four out of fifteen character states is unclear. Indeed for only one state is the polarity defined by information from the fossil record, and two other characters appear to be related to depth distribution rather than to evolutionary history. Although cladistics undoubtedly has a role to play in the elucidation of octopodid phylogenies, it is of limited use until primitive states can be better defined, perhaps by ontogeny, or by looking at higher level relationships within the class Cephalopoda.

At a lower level of taxonomy, in order to understand why there might be so many similar species of *Pareledone* in the Southern Ocean, it is necessary to understand how species arise. It is widely accepted that there are two general

methods of speciation: allopatric and sympatric. Some biologists question the occurrence of the latter (e.g. Felsenstein, 1981; Mayr, 1993) and conclusions of sympatric speciation from a variety of studies have later been disproved with molecular methods (reviewed by Avise, 1994). Grant and Grant (1986) found some evidence for ecological separation of two groups of sympatric Darwin's Finches but this did not persist. Drought initiated the disruptive selection that caused subdivision but random mating eliminated subdivision when normal environmental conditions returned. They concluded that "niches or habitats to which different members of the population adapt should be markedly different and display a long-term persistence, although not necessarily a constancy" if sympatric divergence is to lead to speciation. Lynch (1981) examined vertebrate speciation in a selection of fish, frogs and birds and concluded that sympatric speciation accounted for only 6 % of speciation events. If sympatric speciation does occur it is likely to be confined to a minority of cases.

Allopatric or vicariant speciation occurs when there is geographic separation of two populations of a species. This separation may be a definable barrier such as the Isthmus of Panama (see below) or simply 'isolation by distance' (Wright, 1943), whereby the range of a species is greater than the distance over which populations can maintain panmixia. Each population is prevented from interbreeding with the other and both evolve independently until they are no longer capable of interbreeding. Species that have developed in this matter may then re-invade their original territories and co-exist. Examples of this type of speciation can be seen on either side of the Panama Isthmus. Voight (1988) lists seven geminate congeneric pairs of octopus from either side of this isthmus and concludes that these arose through vicariance following the geologically recent closure of the central American seaway. There are also many more popular examples, for example the Galapagos finches. Thirteen extant species and several fossil species in the subfamily Geospizinae are thought to have been generated from a single presumed ancestral species in 600,000 years (Grant, 1986). Similar radiations have been seen, for example, in Caribbean lizards (*Anolis*) (e.g., Roughgarden 1989, Roughgarden and Pascala 1989), and in the

land snails (*Partula*) of the islands of Hawaii, Polynesia and Micronesia (e.g., Murray *et al.*, 1982, 1988, 1991, 1993).

Evidence of adaptive radiation in molluscs is widespread. In Madeira and the Deserta Islands there are over 260 species of snails. Because of the lack of mobility in the land snails the fauna differs between islands and some species are restricted to a single islet. Other species are common to several islands, and closely related species may co-exist on the same island - presumably a result of re-invasion. 90% of the snails in this island group are from one of four families (cf. 10 families in Europe from where the fauna must have invaded), so snail species have radiated into the space provided by absent mainland species (Cook *et al.*, 1990). In fact, 73% of the snail species are endemic to the islands.

Certain biotypes e.g., isolated islands (see above; Carlquist, 1974), mountain peaks (McDonald and Cowling, 1994), thermal vents (Grassle *et al.*, 1985) and the abyssal zone (Grassle and Maciolek, 1992), support a disproportionate number of endemic species. The presence or absence of these biotypes doubtless disrupts the reported general trend for a decrease in endemism with increasing latitude (Gentry, 1986; Stevens 1989). There are, however, many other exceptions to this pattern; for example, Briggs (1966) concluded that in the Southern hemisphere the percentage of endemic sublittoral invertebrates increased steadily southwards, reaching a maximum in the sub-Antarctic islands. The Southern Ocean itself is a biotype likely to support large numbers of endemic species because it has been isolated for many millions of years (see below). During this time its environment has altered dramatically and although there are few reports on the physiological adaptations of other endemic species (Hawksworth and Kalin-Arroyo, 1995), there is a wealth of information on the endemic fish of the Southern Ocean and their adaptation to cold tolerance (Eastman, 1993). It is likely that there have also been adaptations in the physiology of cephalopods in the Southern Ocean and this is currently being explored by workers in Germany.

Of the six genera reported from the Southern Ocean (including the newly diagnosed genus for "*Pareledone*" *polymorpha*; see Chapter 4) four appear to be endemic. In fish, the endemic species are considered generally to be the old indiginous faunal elements. The Artedidraconidae, the Bathydidraconidae and the Channichthyidae (all notothenoids) for example, are thought to have been associated in their early history with the waters of the Antarctic plate, prior to the formation of the Southern Ocean (Andriashev, 1987 cited in Eastman, 1993). For the majority of the last 600 million years, Antarctica was a component of a much larger land mass. In the late Paleozic Era there was a single continent, Pangea, and a single ocean (that was destined to become the Pacific), Pathalassa. Pangea separated into two smaller land masses, Laurisia and Gondwana, and sea floor spreading in the Jurassic Period caused Laurisia to move northward and Gondwana southward. Antarctica (which was part of Gondwana) attained a South Polar position during the late Cretaceous. At this time, South America, New Zealand and Australia, were still joined to Antarctica, although by this time there were large cracks forming in the continent. Australia began to drift northward about 53 million years ago and final separation between Antarctica and all the other continents occurred around the Eocene-Oligocene boundary (38 million years ago). Deep sea conditions have prevailed in the Drake passage however only since the Oligocene-Miocene boundary, hence Antarctica has been isolated for approximately 25 million years (Kennett, 1982). The only shallow water route into the Antarctic now is round the Scotia Ridge (Dell, 1969). Once Antarctica was fully separated from the previously adjoining continents by sea-floor spreading, the Antarctic Circumpolar Current was unrestricted, and this has served as a barrier to both heat flow and gene flow and has isolated Antarctica thermally and genetically.

Like the endemic notothenoids, it is probable that the endemic genus *Pareledone* was associated in its early history with the waters of the Antarctic Plate, prior to the formation of the Southern Ocean. In shallow water in the Southern Ocean there has probably been little competition from other genera: amongst the

modern fauna, it is rare to find any octopus genera other than *Pareledone* above 200 m depth (Chapter 5). In medium depths (200-800 m) other species such as *Megaleledone senoi*, "*Pareledone*" *polymorpha* and *Benthoctopus ?levis* are also found, but *Pareledone* is very much the dominant genus.

It is likely that the early *Pareledone* underwent vicariant speciation due to geological events (e.g., warming in the Pliocene that led to the formation of interglacial fjords; Webb, 1990) as well as allopatric speciation due to isolation by distance. The latter would be facilitated by the large eggs seen in *Pareledone* (Chapter 4), which probably give rise to benthic hatchlings with limited dispersal capabilities (Chapter 6). Isolation by distance appears to have played a role even in Antarctic fish with a short planktonic stage (Eastman, 1993) so the mechanism is highly likely to be pertinent to speciation in the *Pareledone*. Over time, these new species would invade each other's original territories and hence a large number of similar species could be encountered in any one area. Species might remain morphologically similar because of morphological stasis and stabilising selection, even though they had diverged genetically. Later invasions by other octopodid genera with benthic hatchlings would be unlikely as even the depths involved in the Scotia Ridge route (see above) are likely to be too great for many species to traverse (Chapter 6; Allcock *et al*., 1997). Similarly, water movement is such that temperate planktonic hatchlings are unlikely to be swept into the Southern Ocean on oceanic currents. Also, the cold tolerance mechanisms and other physiological adaptations of the *Pareledone* should make them able to outcompete any potential invaders from temperate seas. Indeed physiological experiments upon the temperate species *Eledone cirrhosa*, showed that these animals could not survive at the reduced temperatures found in the Southern Ocean (Daly, 1996); physiological mechanisms would probably similarly preclude the expansion of *Pareledone* into temperate waters, even in the absence of geographic barriers. The mechanisms outlined above should lead to an endemic genus with many closely related sibling species.

Closer examination of some of the island populations of species, e.g., using molecular methods, may reveal the presence of even greater numbers of species. Although electrophoresis has confirmed several new species, with only eight loci scorable because of enzyme degradation, it is not the most suitable technique for persuit of this subject. Had it been possible to resolve a greater number of loci, these might easily have shown fixed differences between populations of the same species; for example, differences in the colour patterning between populations of *Pareledone turqueti* from South Georgia, the Weddell Sea and the Antarctic Peninsula (Chapter 4) might have been confirmed as genetic differences had more loci been available. DNA techniques are being used to settle similar problems in other Antarctic groups e.g., the serolid isopods (C. Held, pers. comm., 1996). DNA is easily extracted from octopus tissue (Allcock *et al.*, in prep) and extraction could be achieved aboard ship. The DNA could be stored in small amounts of alcohol and this would remove the problems associated with moving either frozen specimens or large amounts of alcohol (classified as dangerous goods).

Finally, it should always be borne in mind that a very small proportion of the Southern Ocean has been investigated for the presence of *Pareledone*. Apart from those areas covered by this research, only the Prydz Bay and Fram Bank have been targeted for octopodid research and it is likely that other undiscovered species are present in unsampled regions of the Southern Ocean.

Bibliography

Adam W (1941). Notes sur les céphalopodes XV. Sur la valeur diagnostique de la radule chez les céphalopodes octopodes. *Bull Mus r Hist nat Belg* **17(38)**:1-19.

Allcock A L, Brierley A S, Thorpe J P and Rodhouse P G K (1997). Restricted geneflow and evolutionary divergence between geographically separated populations of the Antarctic octopus *Pareledone turqueti*. *Mar Biol* **129(1)**:97-102.

Ally J R R and Keck S C (1978). A biochemical genetic population structure study of the market squid, *Loligo opalescens*, along the California coast. *Calif Fish Game* **169**: 113-121.

Ambrose R F (1982). Shelter utilisation by the molluscan cephalopod *Octopus bimaculatus*. *Mar Ecol Prog Ser* **7**: 67-73.

Andriashev A P (1987). A general review of the Antarctic bottom fish fauna. *USSR Acad Sci Proc Zool Inst Leningrad* **153**:9-45. [In Russian]

Andriguetto J M Jr and Haimovici M (1988). Effects of fixation and preservation methods on the morphology of a loliginid squid (Cephalopoda: Myopsida). *Am Malacol Bull* **6(2)**:213-217.

Avise J C (1994). Molecular markers, natural history and evolution. Chapman and Hall, New York.

Avise J C and Ayala F J (1976). Genetic differentiation in speciose versus depauperate phylads: evidence from the California minnows. *Evolution* **30**:46-58.

Barbault R and Sastrapradja S (1995). Generation, maintenance and loss of biodiversity. *In*: Heywood V H and Watson R T (Eds). Global Biodiversity Assessment. Cambridge University Press, Cambridge. pp 192-274.

Beaumont A R and Beveridge C M (1984). Electrophoretic survey of genetic variation in *Pecten maximus, Chlamys opercularis, C. varia* and *C. distorta* from the Irish Sea. *Mar Biol, Berlin* **81**:299-306.

Berry S S (1917). Cephalopoda. *Scient Rep Australas antarct Exped. Series C. Zoology and Botany* **4(2)**:5-38, pls 10-14.

Berry S S (1918). Postscript to the Cephalopoda of the Australasian Antarctic Expedition. A sheet unpaged, inserted in *Scient Rep Australas antarct Exped. Series C. Zoology and Botany* **4(2)**.

Bonnaud L, Boucher-Rodoni R and Monnerot M (1994). Phylogeny of decapod cephalopods based on partial 16S rDNA nucleotide sequences. *C r Acad Sci, Paris, Sciences de la vie / Life Sciences* **317**:581-588.

Bonnaud L, Boucher-Rodoni R and Monnerot M (1996). Relationship of some coleoid cephalopods established by 3' end of the 16S rDNA and cytochrome oxidase III gene sequence comparison. *Am Mal Bull* **12**:87-90.

Bonnaud L, Boucher-Rodoni R and Monnerot M (1997). Phylogeny of cephalopods inferred from mitochondrial DNA sequences. *Mol Phylo and Evol* **7**:44-54.

Boucher-Rodoni R, Martin M and Peduzzi R (1995). Preliminary results of a comparative study of cephalopod eye-lens proteins: the sepiolids. *Bull Inst oceanogr Monaco* **16** (special):93-98.

Boucher-Rodoni R and Bonnaud L (1996). Biochemical and molecular approach to cephalopod phylogeny. *Am Mal Bull* **12**:79-85.

Box G E P and Cox D R (1964). An analysis of transformations. *J R Stat Soc Ser B* **26**:211-243.

Boyle P R and von Boletzky S (1996). Cephalopod populations: definition and dynamics. *Phil Trans R Soc. Series B* **351**:985-1002.

Brierley A S, Allcock A L, Thorpe J P and Clarke M R (1996). Biochemical genetic evidence supporting the taxonomic separation of *Loligo edulis* and *Loligo chinensis* (Cephalopoda: Teuthoidea) from the genus *Loligo*. *Mar Biol, Berlin* **127(1)**:97-104.

Brierley A S, Rodhouse P G, Thorpe J P and Clarke M R (1993b). Genetic evidence of population heterogeneity and cryptic speciation in the ommastrephid squid *Martialia hyadesi* from the Patagonian Shelf and Antarctic Polar Frontal Zone. *Mar Biol, Berlin* **116**:593-602.

Brierley A S, Thorpe J P, Clarke M R and Martins H R (1993a). A biochemical investigation of the population structure of *Loligo forbesi* Steenstrup, 1856 from the UK and the Azores. *In*: Okutani T (Ed). Recent Advances in Cephalopod Fisheries Biology. Tokai University Press, Japan. pp 61-69.

Brierley A S, Thorpe J P, Pierce G J, Clarke M R and Boyle P R (1995). Genetic variation in the neritic squid *Loligo forbesi* (Myopsida: Loliginidae) in the northeast Atlantic Ocean. *Mar Biol, Berlin* **122**:79-86.

Brierley A S (1992). Aspects of genetic diversity and population structure in squid. Unpublished PhD thesis. University of Liverpool.

Brierley A S and Thorpe J P (1994). Chemical genetic evidence supporting the taxonomic separation of *Loligo gahi* from the genus *Loligo*. *Antarct Sci* **6(2)**:143-148.

Briggs J C (1966). Oceanic islands, endemism and marine paleotemperatures. *Syst Zool* **15**:153-163.

Carlquist S (1974). Island biology. Columbia University Press, New York.

Carson H L and Templeton A R (1984). Genetic revolutions in relation to speciation phenomena: the founding of new populations. *Annu Rev Ecol System* **15**:97-131.

Carvalho G R, Thompson A and Stoner A L (1992). Genetic diversity and population differentiation of the shortfin squid *Illex argentinus* in the south-west Atlantic. *J exp mar Biol Ecol* **158**:102-121.

Carvalho G R and Loney K H (1989). Biochemical genetic studies on the Patagonian squid *Loligo gahi* d'Orbigny. I. Electrophoretic survey of genetic variability. *J exp mar Biol Ecol* **126**:231-241.

Charlesworth B, Lande R and Slatkin M (1982). A neo-Darwinian commentary on macro-evolution. *Evolution* **36**:474-498.

Clark A F and Lanigan C M S (1993). Prospects for estimating nucleotide divergence with RAPDs. *Mol Biol and Evol* **10(5)**:1096-1111.

Clarke M R (1962). The identification of cephalopod "beaks" and the relationship between beak size and total body weight. *Bull Br Mus nat Hist (Zool)* **8**:421-480.

Clarke M R (1980). Cephalopoda in the diet of sperm whales of the southern hemisphere and their bearing on sperm whale biology. *Discovery Reps* **xxxvii**:1-324.

Clarke M R (1986). A handbook for the identification of cephalopod beaks. Oxford University Press, London and New York.

Clarke M R (1996). Cephalopods as prey. III. Cetaceans. *Phil Trans R Soc. Series B* **351**:1053-1065.

Cockerham C C and Weir B S (1993). Estimation of gene flow from F-statistics. *Evolution* **47**: 855-863.

Cook L M, Cameron R A D and Lace L A (1990). Land snails of eastern Madeira: speciation, persistence and colonization. *Proc R Soc Lond. Series B* **239**:35-79.

Cook L M (1991). Genetics and ecological diversity. Chapman and Hall, London.

Côrte-Real H B S M, Hawkins S J and Thorpe J P (1996). Population differentiation and taxonomic status of the exploited limpet *Patella candei* in the Macaronesian Islands (Azores, Madeira, Canaries). *Mar Biol, Berlin* **125(1)**:141-152.

Croxall J P and Prince P A (1996). Cephalopods as prey. I. Seabirds. *Phil Trans R Soc. Series B* **351**:1023-1043.

Cuvier G (1797). Tableau élémentaire de l'histoire naturelle es animaux. Baudouin. Paris.

Daly H I (1996). Ecology of the Antarctic octopus *Pareledone* from the Scotia Sea. Unpublished PhD Thesis. Aberdeen University.

Daly H I and Rodhouse P G (1994). Comparative morphology of two sympatric *Pareledone* species from South Georgia. *Antarct Sci* **6**: 163-169.

Dando P R, Storey K B, Hochachka P W and Storey J M (1981). Multiple dehydrogenases in marine molluscs: electrophoretic analysis of alanopine dehydrogenase, strombine dehydrogenase, octopine dehydrogenase and lactate dehydrogenase. *Mar Biol Letts* **2**:249-257.

Dell R K (1959). Cephalopoda. *Rep B A N Z Antarctic Res Exped. Series B* **8(4)**:89-105.

Dell R K (1969). Benthic Mollusca. *In*: Bushnell V C and Hedgpeth J W (Eds). Antarctic map folio series. Folio 11. American Geographical Society, New York.

Dewitt H H, Heemstra P C and Gon O (1990). Nototheniidae. *In*: Gon O and Heemstra P C (Eds). Fishes of the Southern Ocean. J L B Smith Institute of Ichthyology, Grahamstown. pp 279-331.

Digby P G N and Kempton R A (1987). Multivariate analysis of ecological communities. Chapman and Hall, New York.

Dong Z (1991). On the *Pareledone* (Octopoda, Cephalopoda) from the Southern Ocean. *Studia Marina Sinica* 32:183-186.

Eastman J (1993). Antarctic fish biology. Academic Press, London.

Ehrlich P R and Raven P H (1964). Butterflies and plants: a study of co-evolution. *Evolution* **18**:586-608.

Eldredge N and Cracraft J (1972). Punctuated equilibria: an alternative to phyletic gradualism. *In*: Schopf T J M (Ed). Models in paleobiology. Freeman, Cooper and Co, San Francisco. pp 82-115.

Engeser T S (1988). Fossil 'octopods' - a critical review. *In*: Trueman E R and Clarke M R (Eds). The Mollusca. Volume 10. Evolution. Academic Press, London. pp 203-13.

Engeser T S (1990). Major events in cephalopod evolution. *In*: Taylor P D and Larwood G P (Eds). Major evolutionary radiations. *Syst Assoc. Special Volume* **42**:119-138.

Felsenstein J (1981). Skepticism towards Santa Rosalia, or why are there so few kinds of animals. *Evolution* **35**:124-138.

Fitch W M (1976). Molecular Evolutionary Clocks. *In*: Ayala F J (Ed). Molecular Evolution. Sinauer Associates, Massachusetts. pp 160-178.

Gardner J P A (1996). The *Mytilus edulis* species complex in Southwest England - Effects of hybridization and introgression upon interlocus associations and morphometric variation. *Mar Biol, Berlin* **125(2)**:385-399.

Garthwaite R L, Berg C J and Harrigan J (1989). Population genetics of the common squid *Loligo peali* LeSueur, 1821 from Cape Cod to Cape Hatteras. *Biol Bull mar biol Lab Woods Hole* **177**: 287-294.

Gentry A H (1986). Endemism in tropical vs. temperate plant communities. *In*: Soulé M (Ed). Conservation biology. The science of scarcity and diversity. Sinauer Associates, Massachusetts. pp 153-181.

Gormon G C and Renzi J R (1979). Genetic distance and heterozygosity estimates in electrophoretic studies: Effects of sample size. *Copeia* **1979**:242-249.

Goudet J (1994). FSTAT, a program for IBM PC compatibles to calculate Weir and Cockerham's (1984) estimators of F-statistics (version 1.2). Université de Lausanne, Switzerland.

Gould A A (1852). Mollusca and shells. *U S Explor Exped* **12**:1-510.

Grant P R (1986). Ecology and evolution of Darwin's Finches. Princeton University Press, Princeton.

Grant P R and Grant B R (1989). Sympatric speciation and Darwin's Finches. *In*: Otte D and Endler J A (Eds). Speciation and its consequences. Sinauer Associates, Massachusetts. pp 433-457.

Grassle J F, Brown-Leger L S, Morse-Porteous L, Petrecca R and Williams I (1985). Deep-sea fauna of sediments in the vicinity of hydrothermal vents. *Bull Biol Soc Washington* **6**:443-452.

Grassle J F (1989). Species diversity in deep-sea communities. *TREE* **4**:464-469.

Grassle J F (1991). Deep-sea benthic biodiversity. *Bioscience* **51**:464-469.

Grassle J F and Maciolek N J (1992). Deep-sea species richness: regional and local diversity estimates from quantitative bottom samples. *Am Nat* **139**:313-341.

Greenberg N, Garthwaite R L, Potts D C (1996). Allozyme and morphological evidence for a newly introduced species of *Aurelia* in San Francisco Bay, California. *Mar Biol, Berlin* **125(2)**:401-410.

Hadrys H, Balick M and Shearwater B (1992). Application of RAPD in molecular ecology. *Mol Ecol* **1**:55-63.

Hafner M S, Sudman P D, Villablanca F X, Spradling T A, Demastes J W and Nadler S A (1994). Disparate rates of molecular evolution in cospeciating hosts and parasites. *Science* **265**:1087-1090.

Hafner M S and Nadler S A (1988). Phylogenetic trees support the co-evolution of parasites and their hosts. *Nature* **332**:258-259.

Hageman S J (1991). Approaches to systematic and evolutionary studies of perplexing groups: an example using fenestrate bryozoa. *J Paleontology* **65(4)**:630-647.

Haimovici M (1988). *Eledone gaucha*, a new species of eledonid octopod (Cephalopoda, Octopodidae) from Southern Brazil. *Nautilus, Philad* **102(2)**:82-87.

Hammond P M (1992). Species inventory. *In*: Groombridge B (Ed). Global biodiversity, status of Earth's living resources. Chapman and Hall, London. pp 17-39.

Hanlon R T (1988). Behavioural and body patterning characters useful in taxonomy and field identification of cephalopods. *Malacologia* **29(1)**:247-264.

Hanlon R T and Hixon R F (1980). Body patterning and field observations of *Octopus burryi* Voss, 1950. *Bull mar Sci Gulf Caribb* **30(4)**:749-755.

Harris H and Hopkinson D A (1977). Handbook of enzyme electrophoresis in human genetics. North Holland Publishing Co, Amsterdam.

Hartl D L and Clark A G (1989). Principles of Population Genetics. Second Edition. Sinauer Associates, Massachusetts.

Hawksworth D L and Kalin-Arroyo M T (1995). Magnitude and distribution of biodiversity. *In*: Heywood V H and Watson R T (Eds). Global Biodiversity Assessment. Cambridge University Press, Cambridge. pp 107-191.

Hedrick P (1992). Shooting the RAPDs. *Nature, Lond* **355**:679-680.

Helmuth B, Veit R R and Holberton R (1994). Long-distance dispersal of a sub-Antarctic brooding bivalve (*Gaimardia-trapesina*) by kelp-rafting. *Mar Biol, Berlin* **120**: 421-426.

Hennig W (1996). Phylogenetic systematics. University of Illinois Press, Urbana.

van Heukelem W F (1973). Growth and life-span of *Octopus cyanea* (Mollusca: Cephalopoda). *J Zool, Lond* **169**: 299-315.

Hillis D M and Moritz C (1990). Molecular Systematics. Sinauer Associates, Massachusetts.

Hochberg F G, Nixon M and Toll R B (1992). Octopoda. *In*: Sweeney M J, Roper C F E, Mangold K M, Clarke M R, Boletzky S V (Eds). "Larval" and juvenile cephalopods: a manual for their identification. *Smithson Contr Zool* **513**:213-280.

Holmquist J G (1994). Benthic macroalgae as a dispersal mechanism for fauna - influence of a marine tumbleweed. *J exp mar Biol Ecol* **180**: 235-251.

Hoyle W E (1885). Diagnoses of a new species of Cephalopoda. *Ann Mag nat Hist* **5(19)**:222-236.

Hoyle W E (1910). Mollusca: Cephalopoda. *In*: Schultze L S (Ed). Zoologische und anthropologische Ergenbrusse einer Forschungsreise im westlichen und zentralen Südafrika ausgeführt in den Jahren 1903-1905. **4(1)**. Gustab Fischer, Jena. pp 261-268, pl 5a.

Hoyle W E (1912). The Cephalopoda of the Scottish National Antarctic Expedition. *Trans R Soc Edinb* **48(2)**:273-283.

Ihssen P E, Booke H E, Casselman J M, McGlade J M, Payne N R and Utter F M (1981). Stock identification, materials and methods. *Can J Fish Aquat Sci* **38**:1838-1855.

Ingolfsson A (1995). Floating clumps of seaweed around iceland - natural microcosms and a means of dispersal for shore fauna. *Mar Biol, Berlin* **122**: 3-21.

Jackson J B C and Cheetham A H (1994). Phylogen reconstruction and the tempo of speciation in cheilostome Bryozoa. *J Paleobiology* **20(4)**:407-423.

Jeffrey C (1989). Biological nomenclature. Arnold, London.

Jeremiah S J, Povey S, Burley M W, Kietly C, Lee M, Spowart G, Carney G and Cook P J L (1982). Mapping studies on human mitochondrial glutamate oxaloacetate transaminase. *Ann Hum Genet* **46**:145-152.

Johannesson K (1988). The paradox of Rockall - why is a brooding gastropod (*Littorina-saxatilis*) more widespread than one having a planktonic larval dispersal stage (*Littorina-littorea*). *Mar Biol, Berlin* **99**: 507-513.

Johnson G B (1973). Enzyme polymorphism and biosystematics: the hypothesis of selective neutrality. *A Rev Ecol Syst* **4**:93-116.

Jokiel P L (1989). Rafting of reef corals and other organisms at Kwajalein Atoll. *Mar Biol, Berlin* **101**:483-493.

Joubin M L (1905). Description de deux Eledones provenant de l'Expedition du Dr. Charcot dans l'Antartique. *Mém Soc zool Fr* **18**:22-31.

Joubin M L (1906). Céphalopodes. *In*: Expédition Antarctique Française (1903-1905). Sciences Naturelles: Documents Scientifiques. Masson, Paris. pp 1-12.

Joubin M L (1914). Céphalopodes. *In*: Deuxième Expédition Antarctique Française (1908-1910). Sciences Naturelles: Documents Scientifiques. Masson, Paris. pp 35-38.

Katayama T, Wada H, Furuya H, Satoh N, Yamamoto M (1995). Phylogenetic position of the dicyemid mesozoa inferred from 18S rDNA sequences. *Biol Bull* **189(2)**:81-90.

Kayes R J (1974). The daily activity pattern of Octopus vulgaris in a natural habitat. *Mar Behav Physiol* **2**: 337-343.

Kennett J P (1982). Marine Geology. Prentice-Hall, New Jersey.

Kimura M (1983). The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge.

Kimura M and Ohta T (1971). Protein polymorphism as a phase of molecular evolution. *Nature, Lond* **229**:467-469.

Kruckeberg A R and Rabinowitz D (1985). Biological aspects of endemism in higher plants. *A Rev Ecol Syst* **16**:447-479.

Kubodera T and Okutani T (1986). New and rare cephalopods from the Antarctic waters. *Mem natn Inst Polar Res. Special Issue* **44**:129-141.

Kubodera T and Okutani T (1994). Eledonine octopods from the Southern Ocean: systematics and distribution. *Antarct Sci* **6(2)**:205-214.

Kuehl S (1988). A contribution to the reproductive biology and geographical distribution of Antarctic Octopodidae (Cephalopoda). *Malacologia* **29(1)**:89-100.

Lafay B, Smith A B and Christen R (1995). A combined morphological and molecular approach to the phylogeny of the asteroids (Asteroidea: Echinodermata). *Syst Zool* **44(2)**:190-208.

Lamarck J B (1798). Extrait d'un memoire sur le genre de la seche, du calmar et du poulpe, vulgairement nommes, polypes de Mer. *Bull Sci Soc Phil* **2(5)**:129-131.

Larson A, Wake D B and Yanev K P (1984). Measuring gene flow among populations having high levels of genetic fragmentation. *Genet Princeton* **106**: 293-308.

Larson A (1984). Neontological inferences of evolutionary pattern and process in the salamander family Plethodontidae. *Evol Biol* **17**:119-217.

Lasserre P (1992). The role of biodiversity in marine ecosystems. *In*: Solbrig O T, van Emden H M and van Oordt P G W J (Eds). Biodiversity and global change. International Union of Biological Sciences. pp 105-130.

Ledig F T and Cronkle M T (1983). Gene diversity and genetic structure in a narrow endemic, Torrey pine (*Pinus torreyana* Parry ex Carr). *Evolution* **37**:79-85.

Lemen C A and Freeman P W (1989). Testing macroevolutionary hypotheses with cladistic analysis: evidence against rectangular evolution. *Evolution* **43**:1538-1554.

Levins R (1968). Evolution in changing environments. Princeton University Press, Princeton.

Levins R and MacArthur R (1966). The maintenance of genetic polymorphism in a spatially heterogeneous environment: variations on a theme by Howard Levene. *Am Nat* **100**: 585-589.

Levy J A, Haimovici M and Conceico M B (1988). Genetic evidence for two species to the genus *Eledone* (Cephalopoda: Octopodidae) in south Brazil. *Comp Biochem Physiol* **90B(2)**:275-277.

Lewontin R C (1974). The genetic basis of evolutionary change. Columbia University Press, New York.

Lu C C and Stranks T N (1991). *Eledone palari*, a new species of octopus (Cephalopoda: Octopodidae) from Australia. *Bull mar Sci Gulf Caribb* **49(1-2)**:73-87.

Lu C C and Stranks T N (1994). Synopsis of *Pareledone* and *Megaleledone* species, with description of two new species from East Antarctica (Cephalopoda: Octopodidae). *Mem natn Mus Vict* **54**:221-242.

Lynch J D (1981). The gauge of speciation. *In*: Otte D and Endler J A (Eds). Speciation and its consequences. Sinauer Associates, Massachusetts. pp 527-553.

Lynch M and Milligan B G (1994). Analysis of population genetic structure with RAPD markers. *Mol Ecol* **3**:91-99.

Margulis L and Schwartz K V (1988). Five kingdoms: an illustrated guide to the phyla of life on earth. Freeman, New York.

Marshall B A (1996). A new subfamily of the Addisoniidae associated with cephalopod beaks from the tropical southwest pacific, and a new pseudococculinid associated with chondrichthyan egg cases from New Zealand (Mollusca, Lepetelloidea). *Veliger* **39(3)**:250-259.

Massy A L (1916). Mollusca. Part II. Cephalopoda. *Br Antarct Terra Nova Exped 1910 (zool)* **2(7)**:141-175.

Mastro E, Chow V and Hedgecock D (1982). *Littorina scutulata* and *Littorina plena*: sibling species status of two prosobranch gastropod species confirmed by electrophoresis. *Veliger* **24**:239-246.

Mather J A, Resler S and Cosgrove J (1985). Activity and movement patterns of *Octopus dofleini*. *Mar Behav Physiol* **11**: 301-314.

May R M (1994). Biological diversity: differences between land and sea. *Phil Trans R Soc. Series B* **343**:105-111.

Mayr E (1963). Animal species and evolution. Harvard University Press, Massachusetts.

McDonald D J and Cowling R M (1994). Towards a profile of an endemic mountain fynbos flora: implications for conservation. *Biol Conserv* **72**:1-12.

McGowan J A and Walker P W (1993). Pelagic diversity patterns. *In*: Ricklefs R E and Schluter D (Eds). Species diversity in ecological communities: historical and geographical perspectives. University of Chicago Press, Chicago. pp 203-214.

Minelli A (1993). Biological systematics: the state of the art. Chapman and Hall, London.

Morris S C (1995). A new phylum from the lobster lips. *Nature, Lond* **378**:661-662.

Mukai T and Cockerham C C (1977). Spontaneous mutation rates at enzyme loci in *Drosophila melanogaster*. *Proc natn Acad Sci U S A* **74**: 2514-2517.

Murphy P G (1978). *Collisella austrodigitalis* sp. nov.: a sibling species of limpet (Acmaeidae) discovered by electrophoresis. *Biol Bull mar biol Lab Woods Hole* **155**:193-206.

Murphy R W, Sites J W, Buth D G and Haufler C H (1990). Proteins I: Isozyme electrophoresis. *In*: Hillis D M and Moritz C (Eds). Molecular Systematics. Sinauer Associates, Massachusetts. pp 45-146.

Murray J, Clarke B and Johnson M (1993). Adaptive radiation and community structure of *Partula* on Moorea. *Proc R Soc Lond. Series B* **254**:205-211.

Murray J, Johnson M S and Clarke B (1982). Microhabitat differences among genetically similar species of *Partula*. *Evolution* **36**:316-325.

Murray J, Murray E, Johnson M S and Clarke B (1988). The extinction of *Partula* on Moorea. *Pacif Sci* **42**:150-153.

Murray J, Stine O C and Johnson M S (1991). The evolution of mitochondrial-DNA in *Partula*. *Heredity* **66**:93-104.

Naef A (1923). Die Cephalopoden. *Fauna Flora Golf Neapel* **35**:149-836.

Nei M, Fuerst P A and Chakraborty R (1976). Testing the neutral mutation hypothesis by distribution of single locus heterozygosity. *Nature, Lond* **262**:491-493.

Nei M, Fuerst P A and Chakraborty R (1978). Subunit molecular weight and genetic variability of proteins in natural populations. *Proc Nat Acad Sci USA* **75**:3359-3361.

Nei M (1972). Genetic distance between populations. *Am Nat* **106**:283-292.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genet, Princeton* **23**:341-369.

Nei M (1987). Molecular Evolutionary Genetics. Columbia University Press, New York.

Nevo E, Beiles A and Ben-Shlomo R (1984). The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. *In*: Mani G S (Ed). Evolutionary Dynamics of Genetic Diversity. Springer-Verlag, Berlin. pp 13-213.

Nishiguchi M K, Ruby E G and Mcfall-Ngai M J (1995). Coevolution in a squid-luminous bacterium symbiosis; differentiation of sepiolid squids and their symbionts by molecular systematics and bacterial colonization. *Abstr 12th Intern malacol Congr, Vigo* **1995**:316.

Norman J, Murphy J M, Pierce G and Boyle P R (1994). Preliminary molecular genetic analysis of stock structure in the squid *Loligo forbesi* (Steenstrup). ICES Shellfish Committee C M 1994/K:23 8pp.

Norman M D (1991). *Octopus cyanea* Gray, 1849 (Mollusca: Cephalopoda) in Australian waters: description, distribution and taxonomy. *Bull mar Sci Gulf Caribb* **49(1-2)**:20-38.

Ogden R J, Allcock A L, Watts P C and Thorpe J P (in press). The role of characters derived from beak morphology in octopodid taxonomy. *S Afr J Mar Sci.*

Ohdner N H (1923). Die Cephalopoden. *Further zool Results Swed Antarct Exped* **1(4)**:1-7.

Okutani T (1986). A note on Antarctic benthic mollusks collected witha beam-trawl from Breid Bay by the 25th Japanese Antarctic research expedition. *Mem natn Inst polar Res. Special Issue* **40**:277-287.

Olson S L and Jouventin P (1996). A new species of small flightless duck from Amsterdam Island, Southern Indian Ocean (Anatidae, Anas). *Condor* **98(1)**:1-9.

d'Orbigny A D (1835). Mollusques. Voyage dans l'Amérique Méridionale. Paris et Strasbourg.

d'Orbigny A D (1845). Mollusques vivants et fossiles ou description de totes les espèces de coquilles at de mollusques classées suivant leur distribution geologique at geographique. Paris.

d'Orbigny A D and de Férrusac A (1835-1848). Histoire naturelle générale et particulière des céphalopodes acétabulifères, vivants et fossiles. Paris.

Parker T and Tunnicliffe V (1994). Dispersal strategies of the biota on an oceanic seamount - implications for ecology and biogeography. *Biol Bull mar biol Lab Woods Hole* **187**: 336-345.

Perez-Losada M, Guerra A and Sanjuan A (1995). Allozyme electrophoretic technique and phylogenetic relationships in three species of *Sepia* (Cephalopoda: Sepiidae). *Comp Biochem Physiol Ser B* **114(1)**:11-18.

Pierce G J, Thorpe R S and Hastie L C (1994a). Geographic variation in *Loligo forbesi* in the northeast Atlantic. *Mar Biol, Berlin* **119**: 541-547.

Pierce G J, Hastie L C, Guerra A, Thorpe R S, Howard F G and Boyle P R (1994b). Morphometric variation in *Loligo forbesi* and *Loligo vulgaris*: regional, seasonal, sex, maturity and worker differences. *Fish Res* **21**:127-148.

Pojeta J and Runnegar B (1976). The paleontology of rostroconch mollusks and the early history of the phylum Mollusca. *Geol Surv Proj Pap (US)* **968**:1-88.

Poore G (1993). Marine species richness. *Nature, Lond* **361**:597-598.

Rex M A, Stuart C T, Hessler R R, Allen J A, Sanders H L and Wilson G D F (1993). Global scale latitudinal patterns of species diversity in the deep-sea benthos. *Nature,London* **365**:636-639.

Robson G C (1925). The deep-sea Octopoda. *Proc Zool Soc Lond* **1925**:1323-1356.

Robson G C (1929). A Monograph of the Recent Cephalopoda, Part I. Octopodinae. British Museum (Natural History), London.

Robson G C (1930). Cephalopoda. I. Octopoda. *Discovery Reports* **2**:371-402.

Robson G C (1932). Monograph of the Recent Cephalopoda. Part II. Octopoda (excluding Octopodinae). British Museum (Natural History), London.

Rodhouse P G, Arnbom T R, Fedak M A, Yeatman J and Murray A W A (1992). Cephalopod prey of the southern elephant seal *Mirounga leonina* L. *Can J Zool* **70**: 1007-1015.

Rodhouse P G (1990). Cephalopod fauna of the Scotia Sea at South Georgia: potential for commercial exploitation and possible consequences. *In*: Kerry K R and Hempel G (Eds). Antarctic Ecosystems. Ecological Change and Conservation. Springer-Verlag, Berlin. pp 289-298.

Rogers J S (1972). Measures of genetic similarity and genetic distance. *Studies in Genetics, Univ Texas Publ* **7213**:145-153.

Rohlf F J, Loy A and Corti M (1996). Morphometric analysis of Old World Talpidae (Mammalia: Insectivora) using partial-warp scores. *Syst Biol* **45(3)**:344-362.

Rohner M, Bastrop R, Jurss K (1996). Colonization of Europe by two American genetic types or species of the genus Marenzelleria (Polychaeta: Spionidae). An electrophoretic analysis of allozymes. *Mar Biol, Berlin* **127(2)**:277-287.

Roper C F E, Sweeney M J and Clarke M R (1985). Cephalopoda. *In*: Fischer W and Hureau J C (eds). FAO species identification sheets for fishery pruposes. Southern Ocean (Fishing areas 48. 58 and 88) (CCAMLR Convention Area). Vol 1. FAO, Rome.

Roper C F E and Hochberg F G (1988). Behaviour and systematics of cephalopods from Lizard Island, Australia, based on color and body patterns. *Malacologia* **29(1)**:153-193.

Roper C F E and Mangold K M (1991). *Octopus schultzei* (Hoyle, 1910): a redescription with designation of *Aphrodoctopus* new genus (Cephalopoda: Octopodinae). *Bull mar Sci Gulf Caribb* **49(1-2)**:57-72.

Roper C F E and Voss G L (1983). Guidelines for taxonomic descriptions of cephalopod species. *Mem natn Mus Vic* **44**:13-27.

Roughgarden J D (1989). The Anoles of the Eastern Caribbean: competition, coevolution and plate tectonics. Cambridge University Press, Cambridge.

Roughgarden J D and Pacal S (1989). Taxon cycle among *Anolis* lizard populations: review of evidence. *In*: Otte D and Endler J A (Eds). Speciation and its consequences. Sinauer Associates, Massachusetts. pp 403-432.

Runnegar B and Pojeta J (1974). Molluscan phylogeny: the paleontological viewpoint. *Science* **186**:311-317.

Runnegar B and Pojeta J (1985). Origin and diversification of the Mollusca. *In*: Trueman E R and Clarke M R (Eds). The Mollusca. Volume 10. Evolution. Academic Press, London. pp 1-57.

Ryman N and Utter F (Eds) (1987). Population genetics and fishery management. Washington Sea Grant Program, University of Washington Press, Seattle, Washington.

S A S Institute Inc (1989). Sas user's guide, edition 6. Cary, North Carolina.

Sanjuan A, Perez-Losada M and Guerra A (1996). Genetic differentiation in three *Sepia* species (Mollusca: Cephalopoda) from Galician waters (north-west Iberian Peninsula). *Mar Biol, Berlin* **126**:253-259.

Selander R K (1976). Genetic variation in natural populations. *In*: Ayala F J (Ed). Molecular evolution. Sinauer Associates, Massachusetts. pp 21-45.

Selander R K and Kaufman D W (1973). Genic variability and strategies of adaptation in animals. *Proc Nat Acad Sci USA* **70**:1875-1877.

Shaw C R and Prasad R (1970). Starch gel electrophoresis of enzymes, a compilation of recipes. *Biochem Genet* **4**:297-320.

Shaw P W (1997). Polymorphic microsatellite DNA markers in the Veined Squid *Loligo forbesi*. *Mol Ecol* **6**:297-298.

Slatkin M (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**: 264-279.

Smith P J, Mattlin R H, Roeleveld M A and Okutani T (1987). Arrow squids of the genus *Notodarus* in New Zealand waters: systematics, biology and fisheries. *N Z Jl mar freshw Res* **15**:247-253.

Smith P J (1986). Low genetic variation in sharks (Chondrichthyes). *Copeia* **1**:202-206.

Sneath P H A and Sokal R R (1973). Numerical Taxonomy. W H Freeman, San Francisco.

Solé-Cava A M, Thorpe J P and Kaye G (1985). Reproductive isolation with little genetic divergence between *Urticina felina* and *Urticina eques*. *Mar Biol, Berlin* **85**:275-284.

Solé-Cava A M and Thorpe J P (1989). Biochemical correlates of genetic variation in marine lower invertebrates. *Biochem Genet* **27**:303-312.

Solé-Cava A M and Thorpe J P (1991). High levels of genetic variation in natural populations of marine lower invertebrates. *Biol J Linn Soc* **44**:65-80.

Soulé M (1972). Phenetics of natural populations III. Variations in insular populations of a lizard. *Am Nat* **106**:429-446.

Stehli F G, McAlester A L and Helsley C E (1967). Taxonomic diversity of recent bivalves and some implications for geology. *Bull geol Soc Am* **78**:455-466.

Stein D L, Meléndez R C and Kong I U (1991). A review of Chilean snail fishes (Liparididae, Scorpaeniformes) with descriptions of a new genus and three new species. *Copeia* **1992(2)**:358-373.

Stevens G C (1989). The latitudinal gradient in geographical range: how so many species co-exist in the tropics. *Am Nat* **133**:240-256.

Strathmann R R (1985). Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *A Rev Ecol Syst* **16**: 339-361.

Strauss R E (1985). Evolutionary allometry and variation in body form in the South American catfish genus *Corydoras* (Callichthyidae). *Syst Zool* **34**:381-396.

Swofford D L and Selander R B (1981). BIOSYS-1: a fortran program for comprehensive analysis of electrophoretic data in population genetics and systematics. *J Hered* **72**: 281-283.

Taki I (1961). On two new eledonid octopods from the Antarctic Sea. *J Fac Fish Anim Husb Hiroshima Univ* **3(2)**:297-319.

Taylor J D, Morris N J and Taylor C N (1980). Food specialisation and the evolution of predatory prosobranch gastropods. *Palaeontology* **23**:375-409.

Taylor J D (1981). The evolution of predators in the Late Cretaceous and their ecological significance. *In*: Forey L P (Ed). The evolving biosphere. Cambridge University Press, Cambridge. pp 229-245.

Thayer C W (1983). Sediment-mediated biological disturbance and the evolution of marine benthos. *In*: Tevesz M J S and McCall P L (Eds). Biotic interactions in recent and fossil benthic communities. Plenum Press, New York. pp 480-625.

Thiele J (1920). Die Cephalopoden der Deutschen Südpolar Expedition 1901-1903. *Dt Südpo Exped [1905-1931] (zool)* **8(4)**:431-466, pls 52-55.

Thore S (1945). On the Cephalopoda of professor O. Carlgren's expedition to South Africa in 1935. *K fysiogr Sällsk Lund Förh* **15:**49-57.

Thorpe J P, Havenhand J N and Patterson K R (1986). Report of the consultation of the University of Liverpool (Department of Marine Biology) to the Falkland Islands Development Corporation on stock and species identities of Patagonian Shelf *Illex*. Falkland Islands Government, Port Stanley.

Thorpe J P (1982). The molecular clock hypothesis: Biochemical evolution, genetic differentiation and systematics. *A Rev Ecol Syst* **13**:139-68.

Thorpe J P (1989). Possible effect of interprotein variation in the mean rate of amino acid substitution on the relationship of genetic distance with time since evolutionary divergence. *Biol J Linn Soc* **37**:335-344.

Thorpe J P and Solé-Cava A M (1994). The use of allozyme electrophoresis in invertebrate systematics. *Zool Scr* **23(1)**:3-18.

Todd C D (1985). Reproductive strategies of north temperate rocky shore invertebrates. *In*: Moore P G and Seed R (Eds). Ecology of Rocky Coasts. Hodder and Stoughton, London. pp 203-219.

Toll R B (1991). The supraspecific classification of the Octopodinae (Cephalopoda: Octopoda): a review. *Bull mar Sci Gulf Caribb* **49(1-2)**:668.

van Valen L (1973). Are categories in different phyla comparable? *Taxon* **22**:333-373.

Vermeij G J (1977). The mesozoic marine revolution: evidence from snails, predators and grazers. *Paleobiology* **3**:245-258.

Vermeij G J (1978). Biogeography and adaptation. Havard University Press, Massachusetts.

Vermeij G J (1987). Evolution and Escalation: An ecological history of life. Princeton University Press, Princeton.

Villanueva R, Sanchez P, and Compagno Roeleveld M A (1991). *Octopus magnificus* (Cephalopoda: Octopodidae), a new species of large octopod from the south-eastern Atlantic. *Bull mar Sci Gulf Caribb* **49(1-2)**:39-56.

Voight J R (1988). Trans-panamanian geminate octopods (Mollusca: Octopoda). *Malacologia* **29(1)**:289-294.

Voight J R (1991). Morphological variation in octopod specimens: reassessing the assumption of preservation-induced deformation. *Malacologia* **33(1-2)**:241-253.

Voight J R (1993a). The association between distribution and octopodid morphology: implications for classification. *Zool J Linn Soc* **108(3)**:209-223.

Voight J R (1993b). A cladistic reassessment of octopodid classification. *Malacologia* **35(2)**:343-349.

Voight J R (1993c). The arrangement of suckers on octopodid arms as a continuous character. *Malacologia* **35(2)**:351-359.

Voight J R (1994). Morphological variation in shallow-water octopuses (Mollusca: Cephalopoda). *J Zool, Lond* **232**:491-504.

Voss G L (1964). A note on some cephalopods from Brazil with a description of a new species of octopod: *Eledone massyae*. *Bull mar Sci Gulf Caribb* **14**:511-516.

Voss G L (1967). The biology and bathymetric distribution of deep-sea Cephalopods. *Stud trop Oceanogr* **5**:511-535.

Voss G L (1976). Two new species of octopods of the genus *Graneledone* (Mollusca: Cephalopoda) from the Southern Ocean. *Proc biol Soc Wash* **88**:447-458.

Voss G L (1977). Present status and new trends in cephalopod systematics. *In*: The biology of cephalopods. *Symp Zool Soc Lond* **38**:40-60.

Voss G L (1988a). Evolution and phylogenetic relationships of deep-sea octopods (Cirrata and Incirrata). *In*: Clarke M R and Trueman E R (Eds). The Mollusca. Volume 12. Paleontology and Neontology of Cephalopods. Academic Press, London. pp 253-276.

Voss G L (1988b). The biogeography of the deep-sea Octopoda. *Malacologia* **29(1)**:295-307.

Wallace A R (1878). Tropical nature and other essays. Macmillan, London.

Wanless S, Harris M P and Morris J A (1992). Diving behaviour and diet of the blue-eyed shag at South Georgia. *Polar Biol* **12**: 713-719.

Ward R D (1977). Relationship between enzyme heterozygosity and quaternary structure. *Biochem Genet* **15**:123-127.

Ward R D (1978). Subunit size of enzymes and genetic heterozygosity in vertebrates. *Biochem Genet* **16**:779-785.

Ward R D (1989). Molecular population genetics of marine organisms. *In*: Ryland J S and Tyler P A (Eds). Reproduction, genetics and distribution of marine organisms. Olsen and Olsen, Denmark. pp 235-249.

Webb P-N (1990). The Cenozoic history of Antarctica and its global impact. *Antarct Sci* **2**:3-21.

Weir B S and Cockerham C C (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358-1370.

Williamson P G (1981). Palaeontological documentation of speciation in Cenozoic molluscs from Turana Basin. *Nature, Lond* **293**:437-443.

Williamson P G (1987). Selection or constraint: a proposal on the mechanism for stasis. *In*: Campbell K S W and Day M F (Eds). Rates of evolution. Allen and Unwin, London. pp 129-142.

Winston J E (1992). Systematics and marine conservation. *In*: Eldredge N (Ed). Systematics, ecology and the biodiversity crisis. Columbia University Press, New York. pp 144-168.

Wolff G A and Wormuth J M (1979). Biometric separation of the beaks of two morphologically similar species of the squid family Ommastrephidae. *Bull mar Sci Gulf Caribb* **29(4)**:587-592.

Worcester S E (1994). Adult rafting versus larval swimming - dispersal and recruitment of a botryllid ascidian on eelgrass. *Mar Biol, Berlin* **121**: 309-317.

Wright C A and Southgate V R (1981). Coevolution of digeneans and molluscs, with special reference to schistosomes and their intermediate hosts. *In*: Forey L P (Ed). The evolving biosphere. Cambridge University Press, Cambridge. pp 191-205.

Wright S (1943). An analysis of local variability of flower color in *Linanthus parryae*. *Genet Princeton* **28**: 139-156.

Wright S (1978). Evolution and the Genetics of Populations. Volume 4. Variability in and among Natural Populations. University of Chicago Press, Illinois.

Yeatman J and Benzie J A H (1993). Cryptic speciation in *Loligo* from Northern Australia. *In*: Okutani T (Ed). Recent Advances in Cephalopod Fisheries Biology. Tokai University Press, Japan. pp 641-652.

Yochelson E L, Flower R H and Webers G F (1973). The bearing of the new Late Cambrian monoplacophoran genus *Knightoconus* upon the origin of the Cephalopoda. *Lethaia* **6**:275-309.

Yokawa K (1994). Allozyme differentiation of sixteen species of ommastrephid squid (Mollusca, Cephalopoda). *Antarct Sci* **6(2)**:201-204.

Zdzitowiecki K and White M G (1992). Digenean trematode infection of inshore fish at South Georgia. *Antarct Sci* **4**: 51-55.

Zelditch M L, Fink W L and Swiderski D L (1995). Morphometrics, homology and phylogenetics: quantified characters as synapomorphies. *Syst Biol* **44(2)**:179-189.

Zuckerkandl E and Pauling L (1965). Evolutionary divergence and convergence in proteins. *In*: Bryson V and Vogel H J (Eds). Evolving Genes and Proteins. Academic Press, New York. pp 97-166.