

THE ROLE OF BEAK SHAPE IN OCTOPODID TAXONOMY

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Beak shapes in nine species of Southern Ocean octopodids were measured using seven size-standardized ratios. The results were analysed using principal component analysis and discriminant function analysis and show that beak shape may be used as a taxonomic character to distinguish between genera, but not between species. Stepwise discriminant function analysis indicated that all seven ratios were required to maximize discrimination between beaks. A phenogram constructed from a matrix of Mahalanobis distances differed from a dendrogram produced from genetic data. This suggests that, although useful for discrimination, beak morphology is probably not suitable for constructing phylogenies.

Recognition of discrete taxa is a problem in groups that display a great degree of homeomorphy at low taxonomic levels (Hageman 1991). The problem is exacerbated in the Octopodidae because of the lack of hard parts and the phenotypic plasticity of the soft parts. The beak and the radula are the only hard anatomical structures traditionally used in octopodid classification, and there has been extensive characterization of these features in specific descriptions (Lu and Stranks 1991, Norman 1991, Villanueva *et al.* 1991). However, although the importance of the radula in octopodid taxonomy has been reviewed (Adam 1941) and squid beaks are useful in taxonomy (e.g. Clarke 1962, 1980, 1986, 1996), the role of octopodid beak morphology as a systematic character has not been examined in detail.

Analysis of the lower beak, which is more complex than the upper beak, has been applied to the major cephalopod groups (Clarke and Maddock 1988). Such research has led to the suggestion that, because the cephalopod beak does not appear to be specifically adapted to food type, its shape may reflect evolutionary relationships.

It is possible to identify squid 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, however, that there is a greater degree of homeomorphy in octopodid beaks (Clarke 1986), and Voss (1977) considered beak morphology to be unreliable in octopodid taxonomy. Nevertheless, it is suggested in the guidelines for taxonomic descriptions of cephalopod species that beak morphology should be

included in octopus descriptions (Roper and Voss 1983).

The present study attempts to test the systematic reliability of octopodid beak morphology. Morphometric measurements were taken from beaks dissected from specimens of five congeneric species, and from four other confamilial genera, to test the hypotheses that characters derived from beak morphology are suitable for:

- (i) discriminating between closely related species;
- (ii) the construction of phylogenetic trees.

MATERIAL AND METHODS

Specimens were collected during three sampling programmes. Between October 1992 and January 1995, observers aboard commercial fishing vessels operating in the Falkland Islands' 200-mile zone, using a variety of gear, collected octopodids from the by-catch. Between 4 January and 8 February 1994, the waters around South Georgia and Shag Rocks were surveyed by the Falkland Islands' fishery patrol vessel M.V. *Cordella* using a commercial bottom trawl. Between 5 and 28 February 1996, a benthic survey of the coastal shelf of the Weddell Sea was undertaken by the German research vessel P.S. *Polarstern* using a variety of gear, including Agassiz and bottom trawls.

Five species of the genus *Pareledone* were captured (Table I), as well as two other members of the subfamily Eledoninae, "*Pareledone*" *polymorpha* and *Megaleledone senoi*. "*P.*" *polymorpha* does not belong in the genus *Pareledone* (Lu and Stranks

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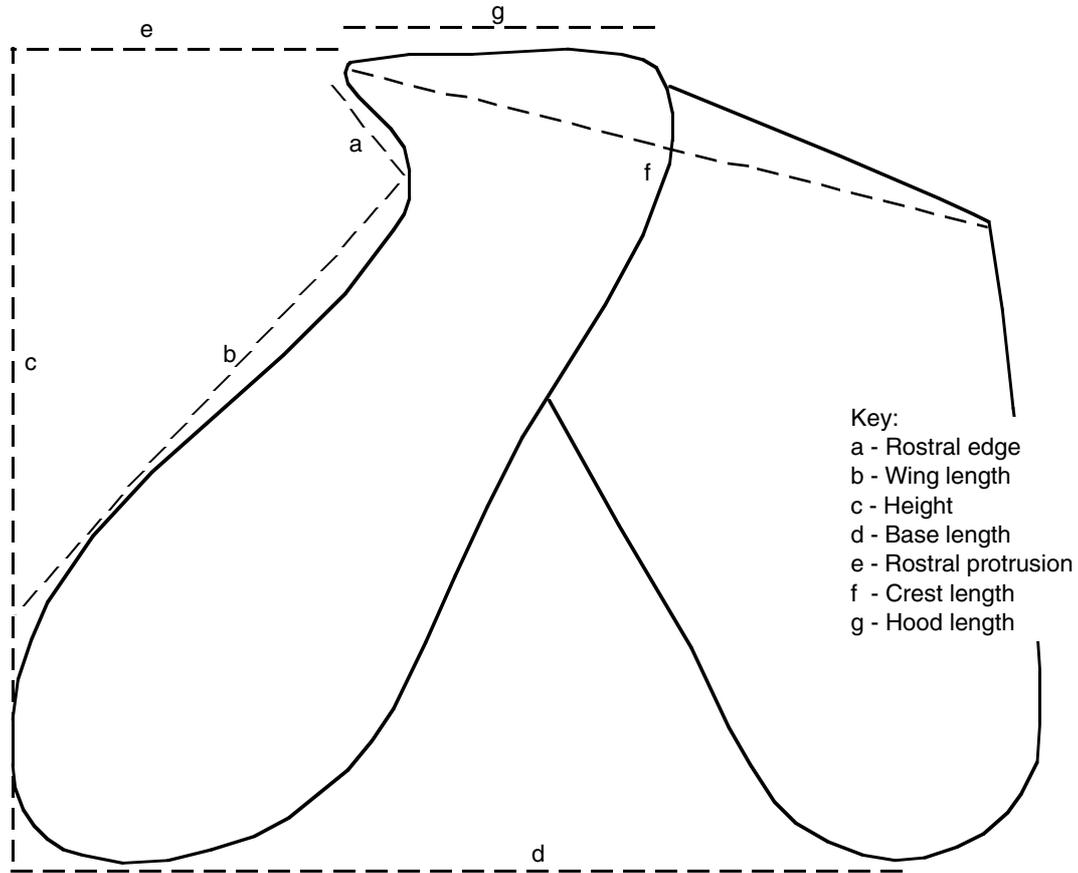


Fig. 1: Diagrammatic representation of an octopodid beak illustrating the seven dimensions measured in this study (adapted from Clarke 1986)

1994, Allcock 1998) and should be placed in a new separate genus (Allcock in prep.). One species representative of the subfamily Bathypolypodinae (*Benthooctopus eureka*) and one species representative of

the subfamily Octopodinae (*Octopus tehuelchus*) were also captured.

Because three of the *Pareledone* species were undescribed (Table I) and because a new genus for

Table I: Number of specimens and general capture location of each species used to test the discriminant power indices derived from beak morphometrics

Species	Number	Capture location
<i>Pareledone turqueti</i>	69	South Georgia/Weddell Sea
" <i>Pareledone</i> " <i>polymorpha</i>	20	South Georgia/Weddell Sea
<i>Benthooctopus eureka</i>	9	Falkland Islands
<i>Octopus tehuelchus</i>	17	Falkland Islands
<i>Megaleledone senoi</i>	10	Weddell Sea
<i>Pareledone</i> sp. 1	29	Weddell Sea
<i>Pareledone</i> sp. 2	21	Weddell Sea
<i>Pareledone charcoti</i>	54	Weddell Sea
<i>Pareledone</i> sp. 3	30	Weddell Sea

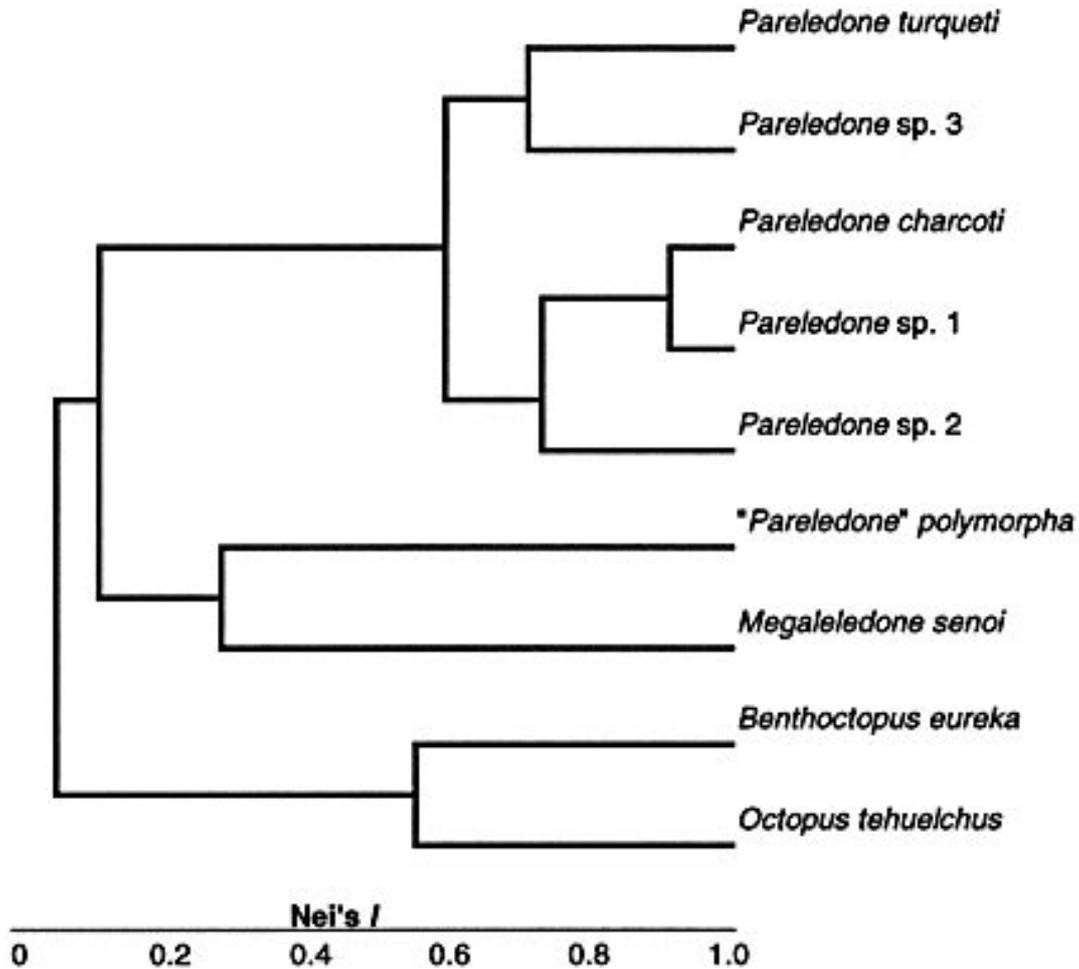


Fig. 2: Analysis of allozyme electrophoretic data from nine species of octopodid. Pairwise squared distances (*I*; Nei 1978) between species clustered using UPGMA

“*Pareledone*” *polymorpha* has not yet been erected, biochemical genetic methods were used to provide evidence for the taxonomic status of each species.

Table II: Description of seven indices derived from morphometric measurements of beaks (see also Fig. 1)

Index	Description
Wing length	Wing length/rostral edge
Height	Height/base length
Rostral protrusion	Base length/rostral protrusion
Hood length	Crest length/hood length
Crest length	Base length/crest length
Rostral edge	Rostral edge/dorsal mantle length
Base length	Base length/dorsal mantle length

Standard procedures for horizontal starch gel electrophoresis (see Allcock *et al.* 1997) were followed and 11 enzyme loci were resolved in each species. Genotype and allele frequencies were analysed using the computer program BIOSYS-1 (Release 1.7, Swofford and Selander 1981). Genetic identity, *I*, (Nei 1978) was clustered using UPGMA (unweighted pair group mean analysis, Sneath and Sokal 1973).

Beaks were dissected from 259 specimens (Table I), cleaned, and stored in ethanol. Measurements were made using image analysis software. Seven dimensions (adapted from Clarke 1986) were recorded (Fig. 1), from which seven indices were calculated (Table II). The resulting data set was analysed using SAS statistical software (SAS Institute Inc. 1988).

Table III: Analysis of seven indices calculated from beak measurements of nine species of octopodid, the resubstitution summary using linear discriminant function. The overall error rate is 36%

Species	Percentage of observations classified into each species									
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	Other
(1) <i>Pareledone turqueti</i>	60.9	–	1.5	–	1.5	–	–	4.4	4.4	27.3
(2) “ <i>Pareledone</i> ” <i>polymorpha</i>	–	90.0	–	–	–	5.0	–	5.0	–	–
(3) <i>Benthoctopus eureka</i>	–	–	88.9	–	–	–	–	–	–	11.1
(4) <i>Octopus tehuelchus</i>	–	–	11.8	82.4	–	–	–	–	–	5.9
(5) <i>Megaleledone senoi</i>	–	–	–	10.0	60.0	–	–	10.0	–	20.0
(6) <i>Pareledone</i> sp. 1	–	3.5	–	–	–	31.0	3.5	13.8	–	48.3
(7) <i>Pareledone</i> sp. 2	–	–	–	–	–	9.5	66.7	–	–	23.8
(8) <i>Pareledone charcoti</i>	5.6	–	–	–	1.9	–	–	79.6	1.9	11.1
(9) <i>Pareledone</i> sp. 3	13.3	–	–	–	–	–	–	16.7	43.3	26.7

To investigate whether beak morphology was useful for discriminating between species, k -nearest neighbour discriminant function analysis (DFA) was used. The value k was selected as the one that minimized the cross-validated error rate (SAS Institute Inc. 1988), although its value is usually non-critical (Hand 1982). Stepwise DFA, a procedure that selects a subset of discriminant variables according to the significance level of an analysis of covariance, was employed on all seven indices to investigate whether any should be excluded from the analysis.

Principal component analysis (PCA) was applied to the data set, and the first three principal components were plotted against one another to illustrate any separation between species.

Mahalanobis (Euclidean) distances were calculated for each pairwise species comparison and clustered using UPGMA. The resulting phenogram was compared with the dendrogram constructed from Nei's I (see above).

RESULTS

The dendrogram constructed from genetic identity values clearly shows that the five species of *Pareledone* (including the three undescribed species) cluster together and separately from all other species (Fig. 2). “*Pareledone*” *polymorpha* does not cluster with this group and is clearly well differentiated genetically from the other *Pareledone* species; this finding supports the suggestion of Lu and Stranks (1994) that this species should be moved to a new genus.

The discriminant function reclassified only 64% of the specimens correctly (Table III). Self-classification was particularly low among species of *Pareledone* (31% in *Pareledone* sp. 1 and 43.3% in *Pareledone* sp. 3), yet it ranged from 82.4 to 90% in *Octopus*,

Benthoctopus and “*Pareledone*”. It is possible that there is little differentiation in beak morphology within genera and that *Pareledone* species were misidentified as one another.

In a principal components analysis of the data from the 259 beaks, the first two eigenvalues were 2.15 and 1.76, accounting for 31 and 25% respectively of the variation in the data. The third eigenvalue was 1.19, accounting for 17% of the variation. The mean scores (and 95% confidence limits) for the first three principal components were plotted for each species (Fig. 3). Although there is good separation between each genus, there is considerable overlap between species of *Pareledone*, supporting the theory that there is little differentiation in beak morphology within genera.

To test this theory further, each specimen was assigned only to genus, and a new discriminant function was calculated. The new function reclassified 88% of the beaks to the correct genus (Table IV). It did not classify any specimens incorrectly, although it was unable to classify a number of specimens. The percentage of *Benthoctopus* and *Megaleledone* specimens that were reclassified correctly was low. This was possibly because there were very few specimens of these genera (9 *Benthoctopus* specimens and 10 *Megaleledone* specimens) available to provide data to create the function.

Stepwise discriminant analysis did not exclude any of the indices from the data set, implying that all the indices were of use in compiling the above discriminant function.

A phenogram constructed from pairwise comparisons of Mahalanobis distance clustered all five species of *Pareledone* closely (Fig. 4). In contrast to the dendrogram constructed from genetic identity values, *Megaleledone* was associated with *Pareledone*, and *Octopus tehuelchus* and *Benthoctopus eureka* were more closely associated with *Pareledone* than was “*Pareledone*” *polymorpha*.

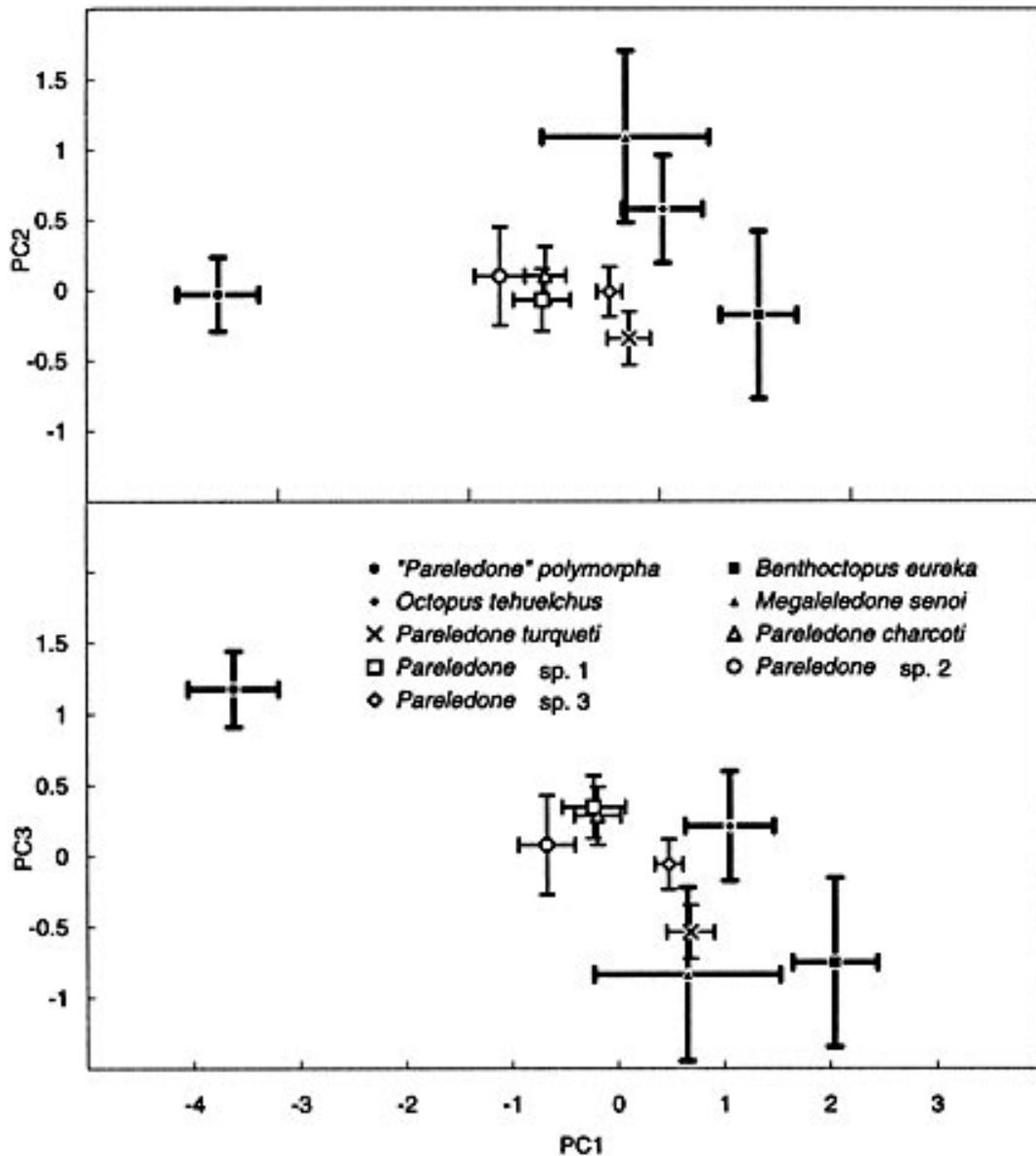


Fig. 3: Principal component analysis on seven indices calculated from beak measurements of nine species of octopodid. (a) Mean scores and 95% confidence limits for the first and second principal components; (b) Mean scores and 95% confidence limits for the first and third principal components

DISCUSSION

Smale *et al.* (1993) have published a key for the

identification of some southern African octopods based on beak characteristics. Thus, beak morphology can be useful in octopod taxonomy. However, few of the species they examined were closely related, and

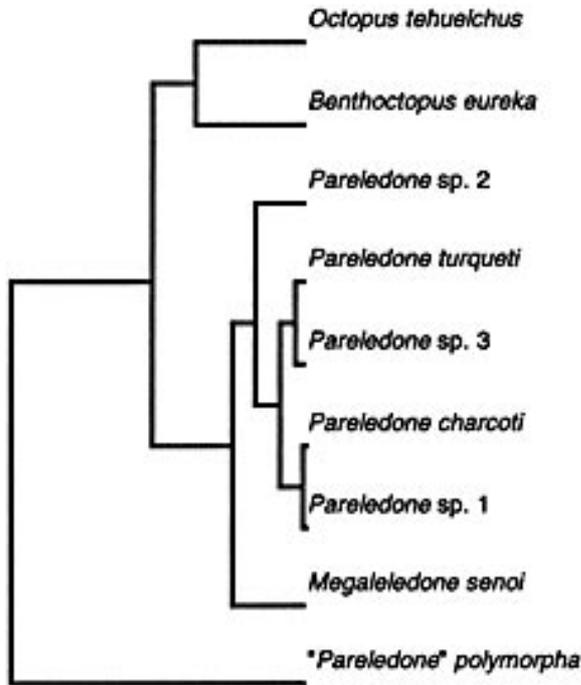


Fig. 4: Analysis of seven indices calculated from beak measurements of nine species of octopodid. Pairwise squared distances between species clustered using UPGMA

the resolution afforded at the lowest taxonomic levels was therefore unclear. A study that attempted to discriminate between the beaks of two congeneric species of octopodid was conducted on "*Pareledone*" *polymorpha* and *Pareledone turqueti* by Daly and Rodhouse (1994). At the time of their study, those species were considered to belong to the same genus, and it appeared that octopodid beaks might therefore be useful for discriminating between sibling species. In fact, that study suggested good separation between

genera. This finding is corroborated in the present work, by the inclusion of more species and more genera.

Although significance levels for DFA probabilities do not exist, correct classification probabilities of >80% have been used as evidence to support the *a priori* classification of taxonomic units (Wolff and Wormuth 1976, Hageman 1991, Reid 1991). The 64% of correct classifications that occurred at species level is well below this critical value, whereas the 88% of correct classifications that occurred at genus level is well above it, indicating the usefulness of beak morphology at least at the level of genus.

Stepwise DFA showed that none of the seven variables could be removed without a reduction in the discriminatory power of the beak shape. Similar conclusions regarding the removal of variables from discriminant analysis of morphological data were reached by Hageman (1991). The findings indicate the importance of including as many characters as possible when attempting to distinguish between closely related taxa using morphometric methods. Discrimination would probably have improved if more measurements were taken from each beak, or if recently developed techniques that measure morphology in terms of distortion from an initial landmark (e.g. Zelditch *et al.* 1995, Rohlf *et al.* 1996) were used. However, although a refinement of methodology may prove that discrimination is technically possible at the specific level, the practical limit of beak morphology in octopodid taxonomy is in genus recognition.

Trees constructed from phenetic and genetic data were dissimilar. The genetic tree is only an estimate of the true phylogeny, but it is considered to be more reliable than morphometric analysis (Avisé 1994). In addition, although UPGMA clustering was originally designed for constructing phenograms (Sokal and Michener 1958), it has since been shown to be a robust method of phylogenetic reconstruction using genetic distance data (Nei 1987, Avisé 1994), and it allows direct comparisons between morphometric and genetic trees. In the phenetic tree, *Octopus tehuelchus* and

Table IV: Analysis of seven indices calculated from beak measurements of five genera of octopus, the resubstitution summary using linear discriminant function. The overall error rate is 12%

Genus	Percentage of observations classified into each genus					
	(1)	(2)	(3)	(4)	(5)	Other
(1) <i>Pareledone</i>	95.6	–	–	–	–	4.4
(2) <i>Benthoctopus</i>	–	33.3	–	–	–	66.7
(3) <i>Octopus</i>	–	–	76.5	–	–	23.5
(4) <i>Megaleledone</i>	–	–	–	30.0	–	70.0
(5) " <i>Pareledone</i> "	–	–	–	–	75.0	25.0

Benthoctopus eureka were more closely associated with *Pareledone* than was "*Pareledone*" *polymorpha*. As the former species both have biserial suckers (cf. *Pareledone* spp. and "*Pareledone*" *polymorpha* with uniserial suckers), they would be expected to cluster separately from the other species in the phenogram (as in the dendrogram of genetic identity), were beak morphology a useful character for constructing phylogenies.

The expectation that beak morphology might be a good phylogenetic character was derived from the assumption that feeding habits have not diversified greatly in the Cephalopoda. This is perhaps true for squid feeding in the open ocean, but may not be true for benthic species, whose range of prey items will depend upon type of substratum. Furthermore, "*Pareledone*" species might be adapted to an atypical diet. Daly (1996) commented on a possible connection between the large posterior salivary glands exhibited by members of this genus and the small delicate rostrum. She suggested that "*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 specialized to exploit a non-benthic food source, such as euphausiids in the water column directly above the sea bed. Such a relationship between form and function would clearly render characters derived from beak morphology unsuitable for use in phylogenetics.

Although it appears, then, that in octopodid taxonomy, the role of the beak is limited to genus identification, the ability to recognize octopodid genera by their beaks has application in other fields. Dietary ecologists have collaborated with squid taxonomists and have successfully clarified the diet of mammalian and avian predators from squid beaks found in stomach contents. As octopodid genera are often restricted to limited but differing depth ranges, the identification of octopodid beaks from the stomachs of predators could yield useful information on the feeding depths, as well as the diet, of these predatory species. Similarly, quantitative information on predator stomach contents could help to assess the biomass of octopodid stocks (e.g. Jackson 1995).

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LITERATURE CITED

- ADAM, W. 1941 — Notes sur les Céphalopodes. 15. Sur le valeur diagnostique de la radule chez les Céphalopodes Octopodes. *Bull. Mus. r. Hist. nat. Belg.* **17**(38): 1–19.
- ALLCOCK, A. L. 1998 — The genetics and taxonomy of Southern Ocean Octopodidae, with special reference to the genus *Pareledone*. Ph.D. thesis, University of Liverpool: 236 pp.
- ALLCOCK, A. L. (in preparation) — A review of the genus *Pareledone* (Cephalopoda: Octopodidae) from Western Antarctica.
- ALLCOCK, A. L., BRIERLEY, A. S., THORPE, J. P. and P. G. RODHOUSE 1997 — Restricted gene flow and evolutionary divergence between geographically separated populations of the Antarctic octopus *Pareledone turqueti*. *Mar. Biol.* **129**(1): 97–102.
- AVISE, J. C. 1994 — *Molecular Markers, Natural History and Evolution*. New York: Chapman & Hall: 511 pp.
- CLARKE, M. R. 1962 — The identification of cephalopod "beaks" and the relationship between beak size and total body weight. *Bull. Br. Mus. nat. Hist.* **8**(10): 421–480 + Plates 13–22.
- CLARKE, M. R. 1980 — Cephalopoda in the diet of sperm whales of the southern hemisphere and their bearing on sperm whale biology. "*Discovery*" *Rep.* **37**: 1–324.
- CLARKE, M. R. (Ed.) 1986 — *A Handbook for the Identification of Cephalopod Beaks*. Oxford: Clarendon: xiii + 273 pp.
- CLARKE, M. R. 1996 — Cephalopods as prey. 3. Cetaceans. *Phil. Trans. R. Soc.* **351B**: 1053–1065.
- CLARKE, M. R. and L. MADDOCK 1988 — Beaks of living coleoid Cephalopoda. In *The Mollusca. 12. Paleontology and Neontology of Cephalopods*. Clarke, M. R. and E. R. Trueman (Eds). San Diego: Academic Press: 123–131.
- CROXALL, J. P. and P. A. PRINCE 1996 — Cephalopods as prey. 1. Seabirds. *Phil. Trans. R. Soc.* **351B**: 1023–1043.
- DALY, H. I. 1996 — Ecology of the Antarctic octopus *Pareledone* from the Scotia Sea. Ph.D. thesis, University of Aberdeen: 169 pp.
- DALY, H. I. and P. G. RODHOUSE 1994 — Comparative morphology of two sympatric *Pareledone* species from South Georgia. *Antarctic Sci.* **6**(2): 163–169.
- HAGEMAN, S. J. 1991 — Approaches to systematic and evolutionary studies of perplexing groups: an example using fenestrate bryozoa. *J. Paleontol.* **65**(4): 630–647.
- HAND, D. J. 1982 — *Kernal Discriminant Analysis*. New York: Research Studies Press: 215 pp.
- JACKSON G. D. 1995 — The use of beaks as tools for biomass estimation in the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae) in New Zealand waters. *Polar Biol.* **15**(1): 9–14.
- LU, C. C. and T. N. STRANKS 1991 — *Eledone palari*, a new species of octopus (Cephalopoda: Octopodidae) from Australia. *Bull. mar. Sci.* **49**(1–2): 73–87.
- LU, C. C. and T. N. STRANKS 1994 — Synopsis of *Pareledone* and *Megaleledone* species, with description of two new species from East Antarctica (Cephalopoda: Octopodidae). *Mem. Mus. Victoria* **54**: 221–242.

- NEI, M. 1978 — Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.
- NEI, M. 1987 — *Molecular Evolutionary Genetics*. New York; Columbia University Press: 512 pp.
- NORMAN, M. D. 1991 — *Octopus cyanea* Gray, 1849 (Mollusca: Cephalopoda) in Australian waters: description, distribution and taxonomy. *Bull. mar. Sci.* **49**(1–2): 20–38.
- REID, A. 1991 — Taxonomic review of the Australian Rossiinae (Cephalopoda: Sepiolidae), with a description of a new species, *Neorossia leptodons*, and redescription of *N. caroli* (Joubin, 1902). *Bull. mar. Sci.* **49**(3): 748–831.
- ROHLF, F. J., LOY, A. and M. CORTI 1996 — Morphometric analysis of Old World Talpidae (Mammalia: Insectivora) using partial-warp scores. *Syst. Biol.* **45**(3): 344–362.
- ROPER, C. F. E. and G. L. VOSS 1983 — Guidelines for taxonomic descriptions of cephalopod species. *Mem. natn. Mus. Victoria* **44**: 49–63.
- SAS INSTITUTE INC. 1988 — *SAS/STAT User's Guide, Release 6.03 Edition*. Cary, North Carolina: SAS Institute Inc.: 1028 pp.
- SMALE, M. J., CLARKE, M. R., KLAGES, N. T. W. and M. A. C. ROELEVELD 1993 — Octopod beak identification – resolution at a regional level (Cephalopoda, Octopoda: southern Africa). *S. Afr. J. mar. Sci.* **13**: 269–293.
- SNEATH, P. H. A. and R. R. SOKAL 1973 — *Numerical Taxonomy. The Principles and Practice of Numerical Classification*. San Francisco; Freeman: xv + 573 pp.
- SOKAL, R. R. and C. D. MICHENER 1958 — A statistical method for evaluating systematic relationships. *Kans. Univ. Sci. Bull.* **38**: 1409–1438.
- SWOFFORD, D. L. and R. B. SELANDER 1981 — BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Heredity* **72**: 281–283.
- VILLANUEVA, R., SÁNCHEZ, P. and M. A. COMPAGNO ROELEVELD 1991 — *Octopus magnificus* (Cephalopoda: Octopodidae), a new species of large octopod from the southeastern Atlantic. *Bull. mar. Sci.* **49**(1–2): 39–56.
- VOSS, G. L. 1977 — Present status and new trends in cephalopod systematics. *Symp. zool. Soc. Lond.* **38**: 49–60.
- WOLFF, G. A. and J. H. WORMUTH 1979 — Biometric separation of the beaks of two morphologically similar species of the squid family Ommastrephidae. *Bull. mar. Sci.* **29**(4): 587–592.
- ZELDITCH, M. L., FINK, W. L. and D. L. SWIDERSKI 1995 — Morphometrics, homology, and phylogenetics: quantified characters as synapomorphies. *Syst. Biol.* **44**(2): 179–189.