

A REVIEW OF AMMONIA-MEDIATED BUOYANCY IN SQUIDS (CEPHALOPODA: TEUTHOIDEA)

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Some deep water squids are known to achieve neutral buoyancy by storing ammonium in their body tissues. The Cranchiidae use a unique coelomic cavity to store ammoniacal fluid; in 15 other families, ammonium appears to be sequestered in either vacuoles in the active body tissues or in a gelatinous outer layer. The hypothesis that these squids form a single lineage is here reconsidered through reviews of morphological characters that could support this hypothesis and physiological mechanisms that could contribute to the repeated evolution of ammonium storage. No readily apparent character identifies all ammoniacal squids as belonging to a single lineage, although 5 families of tissue ammoniacal squids appear to be monophyletic. If the elaborate funnel locking apparatus of this group arose within the clade, it is not homologous with that in other taxa, refuting a basis on which close relationships were suggested for ammoniacal squids. Given the limited data available, we question whether some squids considered to be ammoniacal may develop these characters as a result of senescence. Squids are all thought to derive energy from amino acid catabolism, produce large quantities of ammonia and have very low blood pH's which effectively remove ammonia from cells that produce it. They also minimize H⁺ ion transfer between muscle cells and blood. These features may be important preadaptations for ammonium storage. Given the physiological data, and the absence of morphological evidence supporting monophyly, we argue that ammoniacal squids be best considered to be a polyphyletic group, evolved in parallel.

KEY WORDS: buoyancy, ammonia, evolution, polyphyly

INTRODUCTION

Neutral buoyancy is thought to be advantageous to open water animals by eliminating their need to actively maintain their position in the water column. Most buoyant squids use their body tissues to sequester sufficient levels of ammonia, a cellular toxin, in a low density solution to achieve lift (Clarke *et al.*, 1979). Eight oceanic squid families have been documented as possessing fluid compartments containing ammonium in excess of 200 mmol/l, more than ten times the LD₅₀ for rats (Christensen and Luginbyhl, 1974); members of eight additional families have been suggested to be ammoniacal, apparently due to the consistency of their tissues (Table I).

When ammonium storage was discovered in cranchiid squids, the buoyancy mechanism had been formally reported only in a protozoan (Goethard and Heinsius, 1892, cited by Denton *et al.*, 1969), deep sea shrimps are now known to use ammoniacal buoyancy also (Sanders and Childress, 1988). The evolution of ammoniacal buoyancy in these diverse animals reveals convergence and questions the accuracy with which this feature could reflect evolutionary history.

Tissue storage of ammonium has been hypothesized to have evolved only once in squids, meaning that all squids with this buoyancy mechanism are monophyletic (Clarke, 1988b; Roeleveld and Lipinski, 1991). Here, we examine this hypothesis.

Table 1 Squid taxa currently considered to be ammoniacal. Reported are the taxa that have been documented, or suggested to be ammoniacal, the form of the funnel locking apparatus in the family; the concentration of ammonium in mM if known and morphological basis for classification as ammoniacal with reference. Funnel lock cartilage apparatus as in Roper *et al.* (1969), S = straight; S* = tends to be broad and shortened (Roper, 1969; Young and Roper, 1969b); R = round or triangular; O= oval with projecting inward knobs, F= fused. Ammonium concentration reported by Clarke *et al.* (1979) indicated by *, from Lipinski and Turoboyski (1983) indicated by **, from Robison (1989) indicated by ***, no report indicated by-. Families in bold capitals are thought to belong to a single clade.

Taxon	Funnel lock	[NH ₄]	Modifications, reference
Cranchiidae	F	676**	Coelomic structure (Denton <i>et al.</i> , 1969)
Histioteuthidae	S*	503*	Reticulated mantle & arms (Denton, 1974; Clarke <i>et al.</i> , 1979; Roper and Lu, 1990)
Octopoteuthidae	S*	500**	Outer gelatinous layer and reticulated mantle muscles (Clarke, 1967; Denton, 1974; Clarke <i>et al.</i> , 1979)
Ancistrocheiridae	S	344**	Odor in response to base solution (Clarke <i>et al.</i> , 1979)
Lepidoteuthidae	S	172**	Vacuolated mantle (Clarke <i>et al.</i> , 1979) and dermal cushions (Roper and Lu, 1990)
Architeuthidae	S	338*	Ammonia in mantle and arm (Clarke <i>et al.</i> , 1979)
MASTIGOTEUTHIDAE	O	555**	Ammonia in liquid expressed from tentacle (Robison, 1989)
CHIROTEUTHIDAE	O	276*	Only tentacles non-vacuolated (Dilly <i>et al.</i> , 1977)
JOUBINITEUTHIDAE	R	-	Heavily vacuolated mantle (Clarke <i>et al.</i> , 1979)
PROMACHOTEUTHIDAE	R	-	Heavily invaded with gelatinous tissues (Young and Roper, 1969b)
BATOTEUTHIDAE	R	-	Thick, but gelatinous with 2 weakly developed muscle layers (Roper & Young, 1968) Comparative study (Roper and Lu, 1990)
Bathyteuthidae	S	-	Thin, muscular mantle, like cranchiid (Young and Roper, 1968) Comparative study (Roper and Lu, 1990)
	S*	-	Outer gelatinous layer, thick & muscular mantle wall (Roper, 1969)
		142***	Ammonia in liquid expressed from tentacle (Robison, 1989)
Cycloteuthidae	R	-	Thin gelatinous connective tissue layer between mantle muscles in <i>Cycloteuthis</i> and <i>Discoteuthis discus</i> (the latter with a covering of semi-gelatinous tissue. <i>D. laciniosa</i> with dense mantle and external gelatinous connective tissue (Young and Roper, 1969a)
Neoteuthidae – Otherwise muscular family			
<i>Alluroteuthis</i>	S	-	Based on histological analysis (Filippova, 1991). Comparative study (Roper and Lu, 1990)
Enoploteuthidae – Otherwise muscular family			
<i>Enoploteuthis</i>	S	-	Distal mantle with reticulum of fluid-filled compartments (Roper, 1966). Comparative study (Roper and Lu, 1990)
Onychoteuthidae – Otherwise muscular family			
<i>Kondakovia</i>	S	-	Based on histological analysis (Filippova, 1991).

We review how ammonium is stored by squids and assess whether characters of the mantle, funnel-locking apparatus and gladius are congruent with the evolutionary scenario that these squids are descended from a unique common ancestor. We also assess whether physiological mechanisms exist among squids that may serve as preadaptations for the evolution of ammoniacal buoyancy. Failure to find morphological characters that are congruent with monophyly for tissue ammoniacal squids will not refute the hypothesis, but when strengthened by physiological information, will test this evolutionary model.

THE TAXA

Thirteen families of oceanic squids have been documented, or suggested, to use ammoniacal buoyancy, as have genera in three of the fourteen otherwise non-ammoniacal, muscular families (Table 1). Most of these taxa are comparatively little known, being rarely collected occupants of deep waters (Roper and Young, 1975). Like other cephalopods, these squids are thought to be predators, feeding almost exclusively on protein, deriving most of their energy from amino acid catabolism (Lee, this volume), and excreting ammonia.

Compared to other buoyancy mechanisms, ammoniacal buoyancy offers an advantage in that it maintains function at high ambient pressures or regardless of rapid changes in depth (Denton *et al.*, 1969) and results from storage of a waste product rather than energy rich lipids. Few ammoniacal squids, other than histioteuthids, appear to exploit this advantage by undergoing diel vertical migrations (Roper and Young, 1975). Rather, several taxa, e.g. chiroteuthids (Roper and Young, 1975) and some cranchiids (Voss, 1985), undergo ontogenetic descent; subadults descend to depths where mature adults are found.

Higher order relationships among the tissue ammoniacal squids, as among squids in general (Voss and Voss, 1983), are very poorly known. One apparently monophyletic group has been tentatively identified (Roper and Young, 1967; Young and Roper, 1968; Young, 1991) by characters independent of ammoniacal buoyancy, e.g. tentacle club, funnel-locking apparatus, conus, fin and tail structures. These features appear to be uniquely derived, that is appear to be synapomorphies, of a single lineage or clade, consisting of the Chiroteuthidae (including *Grimalditeuthis*), Mastigoteuthidae, Joubiniteuthidae, Promachoteuthidae and Batoteuthidae, all of which have been suggested or documented to store ammonium in their body tissues.

Although these five squid families appear to form a natural group, no presumed synapomorphies are known to unify all ammoniacal taxa. Especially disconcerting to the hypothesis that tissue ammoniacal squids are monophyletic are suggestions (Roper and Lu, 1990; Filippova, 1991) that otherwise muscular families contain genera that are ammoniacal (Table 1). If these suggestions are supported, then either: 1) these families are not monophyletic, but are artificial groups unified by convergent characters; 2) ammoniacal buoyancy has been lost independently at least three times; or 3) ammoniacal buoyancy has evolved repeatedly among the squids.

ANATOMY OF AMMONIUM STORAGE

Two distinct forms of ammonium storage are found in squids. One family, the Cranchiidae, has undergone unique anatomical modifications to accommodate a specialized coelomic structure dedicated to ammonium storage (Clarke, 1962; Denton *et al.*, 1969; McSweeney, 1978). In the other 15 families of ammoniacal squids (Table 1), ammonium is stored in seemingly intimate contact with normal muscles and nerves (Clarke *et al.*, 1969; also see illustrations in Clarke *et al.*, 1979; Dilly *et al.*, 1977; Roper and Lu, 1990). How the cranchiids relate to the ammoniacal squids has been considered, but remains unresolved (Clarke, 1988a: Figure 1). In contrast to the tissue ammoniacal squids, cranchiids have low concentrations of ammonium in the musculature (Lipinski and Turoboyski, 1983) and appear to have a muscular

hydrostatic skeletal system similar to other cephalopods (Clarke *et al.*, 1979; McSweeney, 1978; Roper and Lu 1990).

The presence of mutually perpendicular muscles in *Nautilus*, squids and octopuses (Kier, 1982; 1987; 1988; Kier and Smith, 1985; Kier and Smith, 1990) indicates that this skeletal system is primitive and highly conserved among cephalopods. One character apparently common among squids is the replacement of the longitudinal mantle muscles by inner and outer sheaths of connective tissue which surround the mutually perpendicular circular and radial muscles (Ward and Wainwright, 1972; Bone *et al.*, 1981; Gosline and DeMont, 1985). Longitudinal mantle muscles have been illustrated schematically in *Lepidoteuthis* (Clarke and Maul, 1962; Clarke, 1964), *Taningia* (Clarke, 1967) and in a photomicrograph of *Cranchia* (Roper and Lu, 1990). These, however, appear to be inconsistencies, as Chun (1910, Plate LIV) illustrates and identifies only two muscle layers in *Cranchia*, as do Roper and Lu (1990) in *Lepidoteuthis*. In spite of these inconsistencies, the conservation of muscular anatomy observed through the Class Cephalopoda strongly argues that once evolved, muscular modifications for ammonium storage would be conserved within a lineage. The two different tissue ammonium storage systems apparent among tissue ammoniacal squids violate this prediction and suggest that ammonium buoyancy has evolved at least twice in this group.

In seven taxa, ammoniacal vacuoles occur within the circular mantle muscles (Table 1). In the Lepidoteuthidae (including *Pholidoteuthis*), vacuolated dermal cushions may store ammonium in addition to the heavily vacuolated mantle tissue (Roper and Lu, 1990). In three taxa, thick layers of gelatinous material lie external to the mantle musculature (Table 1). Where ammonium is stored in the other five taxa is undocumented.

A startling point of apparent conflict is that most members of the Cycloteuthidae have vacuoles in the central mantle muscles but these are absent in specimens of *Discoteuthis laciniosa* (Young and Roper, 1969a). This inconsistency within a genus (*D. discus* shows the typical mantle vacuoles) strongly suggests that the buoyancy mechanism was not present in the common ancestor, but arose by convergence.

MANTLE MUSCLE SPECIALIZATION

Enzyme levels, blood supply, mitochondria distribution and muscle fiber size indicate that the outer and inner muscles of the mantle have high aerobic capacity and the central muscles have low aerobic capacity (Bone *et al.*, 1981; Mommsen *et al.*, 1981). These differences are thought to correspond to the function of the peripheral muscles in normal respiration, and of the central muscles in escape jets. The functional differences in the mantle musculature documented in four families of muscular squids (Mommsen *et al.*, 1981) may be the primitive condition among squids.

That the central muscles specialized for anaerobic function most often appear to serve as ammonium storage sites (Table 1) may not be evidence for common ancestry. Detailed *in vivo* studies of each muscle layer are yet to be attempted, but studies of the pooled mantle muscle cells find that compared to poikilothermic vertebrates (Pörtner *et al.*, 1991a; 1993), muscles of exercised squid appear to retain protons rather than giving them to the blood. This results in a comparatively constant blood pH, and fluctuations in muscle pH's.

Retention of protons by exercised mantle muscles in *Illex* led to a drop of 0.5–0.6 pH units, as measured in the entire mantle. If the observed drop were due to averaging a change that had occurred in only a narrow section of the mantle, the local drop would have been from 1.0 to 1.2 pH units. The sparsity of capillaries in the inner section of the mantle (Bone *et al.*, 1981) makes this area seem especially vulnerable to wide pH shifts. As a corollary, low rates of ion exchange may prevail in this section of the mantle, where, obviously, ammoniacal vacuoles could develop and be readily sealed from other body fluids (Clarke *et al.*, 1979). Specialization of the inner mantle for anaerobic function may therefore preadapt this muscle layer to store ammonium.

PHYSIOLOGY

Observations of live specimens of ammoniacal squids are rare; detailed observations of their metabolic processes are virtually unknown. Recent advances in our knowledge of squid physiology have been largely based on loliginids and ommastrephids. As these families are classified in separate suborders, we base a working hypothesis of the ancestral condition of teuthoids on these data. These commonalities that may pertain to ammoniacal buoyancy include their reliance on nitrogen catabolism for energy and an unusually low blood pH.

The large amounts of proline that cephalopods burn in aerobic metabolism (Storey and Storey, 1983) produce ammonium and bicarbonate ions in close to equimolar amounts, as is common in water-breathing animals (Pörtner, 1989). Rates of ammonia excretion and oxygen consumption are tightly coupled, with O/N ratios of cephalopods typical for a protein diet (Hoeger *et al.*, 1987; Boucher-Rodoni and Mangold, 1989). The rate of ammonia formation in squids, however, is the highest known among invertebrates. Although Denton *et al.* (1969) estimated that cranchiids would have to retain 40% of their life time production of ammonia to achieve neutral buoyancy, ammonium concentrations in more active muscular squids could rise rapidly. If squids retained rather than excreted the ammonia measured by Hoeger *et al.* (1987) for 12 days, their body fluids would reach ammonia concentrations of up to 500 mmol/kg, without requiring any ammonia to be acquired directly, such as from cannibalism or consumption of other ammoniacal species.

The ample ammonia produced must be removed from active cells and, in ammoniacal taxa, stored. Ammonium distribution is strongly linked to pH, as indicated by *in vivo* studies that ensure protection of labile amino acids during experimental analysis (Heisler, 1993). In the muscular squid, *Illex illecebrosus*, (Pörtner *et al.*, 1991a; 1993) the minimal pH differences seen between mantle muscle cells and blood are paralleled by small differences in ammonium concentration (0.5–0.6 mmol/kg, muscle cells, versus 0.1–0.3 mmol/l blood).

The pH of cephalopod blood, 0.4 to 0.5 pH units lower than in other animals at a given temperature (Heisler, 1986), may increase its efficiency in removing ammonia by decreasing levels of free ammonia in the blood. By readily protonating ammonia to form ammonium, low blood pH reduces ammonia partial pressures and the loss of ammonia by non-ionic diffusion. Ammonium is resistant to loss by passive diffusion and is less toxic. At equilibrium, however, high blood concentrations of ammonium may exceed levels necessary to maintain this advantage. Low pH, likely to be a universal character of cephalopod blood, may be critical in the evolution of ammoniacal buoyancy among squids.

Despite the high amounts of ammonia produced and low blood pHs, concentrations of ammonium circulating in the blood of exercised muscular squid are comparable to those of other marine invertebrates, e.g. the 0.2 mmol/l in coelomic plasma of a sipunculan worm (Pörtner *et al.*, 1991b). Because the net pH gradient between the cells and the environment influences the concentration gradient for free ammonia regardless of the pH of the blood, it is the net force driving ammonia excretion. In squids, this pH gradient is comparable to that in other marine invertebrates and poikilothermic vertebrates.

To concentrate sufficient ammonium to achieve neutral buoyancy, Denton (1974) suggested that lowering the pH of isolated fluid compartments would be essential. The tissue ammonium concentrations reported by Denton *et al.* (1969) and Clarke *et al.* (1979) would require that the pH's of ammoniacal fluid to be about 2 units below those of the blood to store enough ammonium to achieve neutral buoyancy. In histioteuthids, vacuolar fluid pH of various vascular tissues was determined to be as low as 5.1 compared to a maximum blood pH of 7.2 (Clarke *et al.*, 1979). However, most reported values differed by a lesser amount. Considering that the analyzed fluids may have been contaminated with ruptured cells and their buffering contents, these differences may be considered estimates at best.

Assuming that measured pH differences that match the predictions are valid, maintaining such a gradient would require a considerable amount of energy. Denton's (1974) statement that the use of a pH gradient to sequester ammonia would require little energy expenditure may be in error. Carrier mechanisms would probably be required to assist in maintaining pH and/or ammonium gradients. Likely candidates for carrier mechanisms in a transport epithelium would be Na^+/H^+ exchangers to replace Na^+ with NH_4^+ to form the ammoniacal fluid and cause pH to fall. Ammonium (instead of H^+) transport by this carrier and back diffusion of unprotonated ammonia would be equivalent to net proton transport until the required pH values were reached to minimize net ammonia flux. An H^+ -ATPase could be a likely carrier if a cellular vacuole were used to store ammonium.

Surrounding ammonium storage compartments with diffusion-reducing epithelia would reduce energy requirements and diffusive flux. Such vacuoles could be derived from specialized fluid compartments in individual cells or from extracellular lacunas surrounded by cells containing tight junctions. As lipid solubility of ammonia is considered to be poor, diffusion would likely occur only through hydrated charged membrane channels resistant to ionic movement, but not to movement of neutral molecules (Heisler, 1993). Restricting the number of such channels would reduce ammonia diffusion.

Ultrastructural studies of ammonium vacuoles are needed to determine whether extra- or intra cellular structures are used, and thereby test whether grossly similar structures are convergent. The high quantities of ammonium that squids produce provides them with ammonium to sequester, the low pH of cephalopod blood is an efficient means of moving ammonium within the body. Alternate mechanisms exist which, at least in theory, could be readily modified to sequester ammonium.

BUOYANCY AND LIFE SPAN

In terms of whole-animal biology, some tissue ammoniacal squids appear to be nearly neutrally buoyant throughout their lives. The weight in water of specimens

of *Histioteuthis* sp. increases fourfold as their weight in air increases over 40-fold (Clarke *et al.*, 1979). Vacuoles occur within the mantle of specimens of *Lepidoteuthis* having mantle lengths of from 6 to 97 cm (Clarke, 1964). However, in the three ammoniacal squid species that have had more than one individual analyzed, the ammonium concentrations vary widely without obvious correlation with the condition of the animal (Lipinski and Turoboyski, 1983).

Determining how ammonium concentrations change and how buoyancy develops over the life span of individuals is critical because the gelatinous tissue consistency often associated with ammoniacal buoyancy develops in muscular squids with reproductive maturity and senescence (Arnold and Williams-Arnold, 1977; McSweeney 1978; Kristensen, 1981; Voss, 1985). Mantle sections of immature females of the cranchiid *Galiteuthis glacialis* show dense muscles in contrast to the vacuolated mantle sections of a gravid female (McSweeney, 1978: Plate 2).

Degeneration of the mantle tissue in diverse families of muscular squids suggests that protein repair mechanisms break down at senescence in squids as in octopuses (O'Dor and Wells, 1978). Voss (1985) postulated that the tissue breakdown in maturing female squids could relate to energetic demands of egg production and to the need for additional buoyancy to counter the increased weight of eggs and reproductive organs. Adam's (1972) report of a dead specimen of the onychoteuthid *Chaunoteuthis mollis* floating on the sea suggests that gelatinous tissue consistency is associated with increased buoyancy in muscular squids. Only flaccid females of this species and of *Gonatopsis japonicus* and *G. okutanii* are known (Okiyama, 1969; Adam, 1972; Nesis, 1972; Murata and Okutani, 1975). Even among ammoniacal squids, some species, e.g. *Histioteuthis elongata* and *H. miranda* are known only from females (Voss, 1969) which, in the case of *H. elongata*, were found floating on the sea surface.

These reports suggest that senescent muscular squids can mimic tissue ammoniacal squids. If senescent squids retain rather than excrete ammonia, the resultant high concentrations in the tissues, and perhaps in the histology of the tissues, may be indistinguishable. With ammonium compartments and ammonium concentrations documented in so few specimens (Table 1) we cannot be certain that each taxon currently considered to be ammoniacal uses this buoyancy mechanism through its life cycle. Some could adopt it in the late stages of the life cycle, perhaps to allow gravid females to fully mobilize body energy reserves for egg production while reducing the risks of predation.

Late adoption of ammoniacal buoyancy is consistent with ontogenetic descent of ammoniacal taxa and with the limited diel vertical migrations in the group (Roper and Young, 1975). Additional research is required to determine whether each taxon uses the lift provided by ammonium through the life cycle or only as senescence approaches.

PHYLOGENETIC RECONSTRUCTION: CURRENT STATUS

If ammoniacal squids are monophyletic then derived characters that reflect their common ancestry should be present. The summary of basic characters of teuthoid families by Roper *et al.* (1969) fails to identify characters that unify all tissue ammoniacal squids. However, except for the ommastrephids and thysanoteuthids, only ammoniacal squids have an elaborate funnel-locking apparatus, the cartilaginous

ridge and groove system that closes the mantle during escape jets. Donovan and Toll's (1988) review of the gladius gives a partial list of taxa with a secondary conus, all of which are ammoniacal.

Both of these structures may relate to increasing the efficiency of escape jets. Elaborating the ridge and groove funnel locking apparatus, considered to be primitive by Young and Roper (1968), may firmly seal the mantle collar to the funnel to increase the force of the water ejected during an escape jet. Elaborating the primary conus may maintain mantle length during a jet, and thus the amount of water expelled (Ward and Wainwright, 1972).

Although these features appear to be most common among ammoniacal squids, analysis of the occurrence of an elaborate funnel locking apparatus and of the secondary conus, using additional data from Toll (1982), demonstrated that neither is significantly more likely to occur among ammoniacal than muscular squids. In addition, the seeming dominance of these characters among ammoniacal squids may be attributed to their presence in most members of the Chiroteuthid clade (for funnel lock, see Table 1). If, for example, the elaborate funnel locking apparatus evolved once, early in the evolution of this clade, that elaboration is unique to that group, and convergent rather than homologous with similar structures in other lineages.

Voss and Voss (1983), recognizing that a complete analysis of the teuthoids was virtually impossible, selected the Promachoteuthidae, the Chiroteuthid clade (except Batoteuthidae), the Cycloteuthidae and the Thysanoteuthidae as outgroups for their phylogenetic analysis of the cranchiids. Voss (1988) withdrew the thysanoteuthids based on characters of the gladius. These taxa were selected because they share a complex funnel locking apparatus and a ventral attachment of the buccal membrane to the fourth arms.

As discussed above, recent research (Young, 1991) strengthens the view that the Chiroteuthid clade shares a more recent common ancestor than do other squids. The complex funnel locking apparatus of this group is not homologous with that in any other squid group. This conclusion leads us to refute the above mentioned outgroups as potential sister groups to the cranchiids. Therefore, the cranchiids with coelomic buoyancy may not be closely related to tissue ammoniacal squids.

Characters argued to support monophyly for the tissue ammoniacal squids (Clarke, 1988a) include statoliths and ammoniacal buoyancy itself. This latter character offers only circular support for this argument and statolith shape varies with swimming mode (Young, 1977; 1984; 1988).

Roeleveld and Lipinski (1991) in discussing the relationships of architeuthids state that it would be difficult for ammoniacal buoyancy to evolve twice. However, Denton (1974: 297) stated that the evolution of ammoniacal buoyancy may only be a small step given the nature of the physiology of cephalopods. If ammoniacal buoyancy evolved only once and these squids are monophyletic, ammoniacal fluid storage mechanisms to have had to have diversified without leaving evidence of a common ancestor, even within a single genus, and muscular families would have to be paraphyletic. The assumption that ammoniacal squids arose independently requires only parallel adaptations of the low blood pH, the anaerobic musculature of the mantle, and perhaps the neotenus adoption of senescent characters. Distinguishing these possibilities could be done through ultrastructural analysis of ammonium compartments, or by phylogenetic analysis of the teuthoids using characters independent of buoyancy to determine whether ammoniacal buoyancy appears once,

or many times. With our current knowledge of their ecology, character variability and ontogeny, it is premature to build phylogenetic trees of the teuthoids, or even to group squids according to buoyancy mechanisms.

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