A REVIEW OF THE PYGMY SQUID *IDIOSEPIUS*: PERSPECTIVES EMERGING FROM AN "INCONSPICUOUS" CEPHALOPOD

M. K. NISHIGUCHI[1*]*, J. NABHITABHATA*[2]*, N. A. MOLTSCHANIWSKYJ*[3]*,
S. V. BOLETZKY*[4]

[1] *New Mexico State University, Department of Biology, Box 30001, MSC 3AF, Las Cruces, NM 88003-8001, USA*
[2] *Centre for Biodiversity of Peninsular Thailand, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla 90112, Thailand*
[3] *The University of Newcastle, School of Environmental and Life Sciences, Ourimbah NSW, 2258, Australia*
[4] *CNRS, Observatoire Océanologique de Banyuls, Av du Fontaulé, 66650 Banyuls-sur-Mer, France*
* *Corresponding author: nish@nmsu.edu*

IDIOSEPIUS
CEPHALOPOD
LIFE HISTORY
EVOLUTION
SQUID
MOLLUSC

ABSTRACT. – The monogeneric family Idiosepiidae (Mollusca: Cephalopoda: Idiosepiida) contains the smallest living representatives of squid. Sexual dimorphism, presence of an adhesive organ on the dorsal mantle integument, lack of fully developed tentacles on hatching, and ability to produce large quantities of eggs relative to their body size during reproduction provide unique characteristics not commonly found in other cephalopods. These "mini-maximalists" have a life history strategy of rapid growth and high fecundity, and species of *Idiosepius* have been used as a model to examine embryonic and post-embryonic development, neurobiology, phylogeny, physiology, and life history strategies. Their small size, rapid generation time, solitary nature, and ease of producing eggs/hatchlings in captivity has provided a solid foundation for better understanding the evolution of an organism that has pushed the boundaries of a multitude of life history characteristics not observed in other metazoans.

INTRODUCTION

Species of the genus *Idiosepius* (Mollusca: Cephalopoda) are very small temperate and tropical squids commonly found in littoral waters in the Indo-Pacific region from South Africa to Japan and southern Australia (Lu & Dunning 1998). They are an ideal model system for laboratory studies due to their short life-span (approximately 80-90 days) and continuous growth throughout their life (Jackson 1989, Tracey *et al*. 2003). It is one of the smallest decabrachians among cephalopod groups (15 mm mantle length; Fig. 1); only few octobrachian relatives are as small in size as an adult *Idiosepius* (Kasugai 2000;

Boletzky 2003). The small adult size (10-18 mm total length), the attachment behavior of adults of *Idiosepius*, and direct development raise questions about the ecology of the species. In particular capacity for dispersal during the lifetime, mobility during the post-hatching planktonic phase, patterns of distribution and the related consequences of biological and ecological constraints, and reproductive strategies allowing continuous growth and enhanced reproductive output. Thus, the Idiosepiidae have a diversity of traits that are interesting from a variety of evolutionary perspectives. While there has been a long history of the use of cephalopod systems being used as models to inform function and evolution of vertebrate systems *e.g.*,
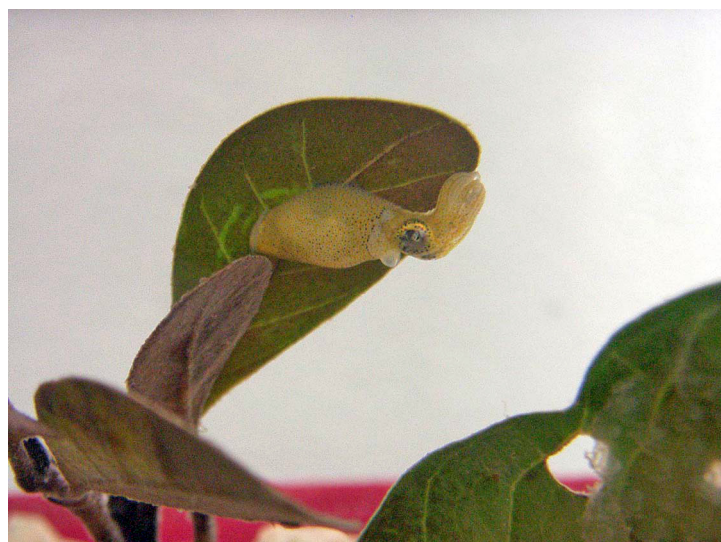

Fig. 1. – *Idiosepius biserialis* from the Andaman Sea, Thailand. The animal sticks to the leaf of an aquarium plant using its dorsal attachment organ; the peculiar position of the arms is exactly as described by Sasaki (1921). Photo courtesy of K. Warnke.

giant axon research and eyes, more recently, *Idiosepius* is being used to explore the evolution of signaling molecules in closed circulatory systems (Yoshida *et al*. 2010a).

There are seven described species of *Idiosepius; I. pygmaeus* Steenstrup, 1881, *I. paradoxus* Ortmann, 1888, *I. picteti* Joubin, 1894, *I. notoides* Berry, 1921, *I. biserialis* Voss, 1962, *I. macrocheir* Voss, 1962, *I. thailandicus* Chotiyaputta, Okutani & Chaitiamvong, 1991, and one undescribed temperate species on the east coast Australia (personal comment Dr A Reid, Australian Museum). Renewed interest in pygmy squid arose half a century ago when Voss (Voss 1962) described two new species of *Idiosepius* (*I. biserialis*, *I. macrocheir*), four decades after the description of *I. notoides* by Berry (Berry 1921). Voss' work brought the number of recognized species to six, three decades before *I. thailandicus* was described (Chotiyaputta *et al*. 1991). The position of *I. minimus* (*c.f.* Jereb & Roper 2005) with the genus was indeed unverified due to the disputed position within the genus. This work, along with studies by Hylleberg and Nateewathana (Hylleberg & Nateewathana 1991b, a) confirmed the presence of a gladius (pen) in *Idiosepius* species, a fact that had been previously disputed. That this family of squids has only one genus with seven described species has motivated malacologists to question the radiation of the family compared to other "cuttlefish-like" squids, with respect to differences in their life history characteristics.

Comparisons between the Idiosepiidae and these related families show many similarities; Sepiidae and Sepiolidae are holobenthic/benthic and mostly found in shallow coastal waters, usually living among sandy bottoms or within seagrass beds, similar to the Idiosepiidae. They have a sedentary lifestyle (except Heteroteuthinae), either resting or burying in or on top of some type of substratum (sand or reef; Sepiidae and Sepiolidae) or as in the case of pygmy squids, adhering to blades of eelgrass and macroalgae with a specialized adhesive organ (which may also reduce energy expenditure; Fig. 2). This characteristic is somewhat similar to that of sepiolid squids, which adhere sand to their mantle. The cryptic behavior of *Idiosepius* spp. decreases the risk of predation since most species are small and are easy prey items (Moynihan 1983a). Most individuals are solitary and rarely seen in large schools like teuthid squids (Moynihan 1983a, b). While all three families are sequential spawners, the small size of the Idiosepiidae represents the physiological edge that most benthic cephalopods can tolerate.

Although the pygmy squids are among the smallest in the entire class of cephalopods, they provide a great deal of information for understanding how a tiny "maximalist" squid can provide detailed information not only to malacologists, but to a wide field of disciplines, ranging from behavior to evolution. This review is a compilation of ideas and research (both published and unpublished) that were brought to our knowledge from a workshop convened at the Cephalopod International Advisory Council meeting held in Phuket, Thailand in February 2003 (Boletzky *et al*. 2005). We have included most of the published works related to *Idiosepius*, as well as "grey" and unpublished literature. The small size, short life span, solitary life, and amenability to laboratory experiments have made it an attractive species for student projects which are lodged in B.Sc. Honors theses, produced at University of Sydney, James Cook University, and the University of Newcastle (Australia) in the last 20 years. Although some studies are limited in time and scope, we felt that they were just as important as the major published research articles, so we have included them in this review. Our goals were to bring together knowledge about all aspects of the pygmy squid (Family Idiosepiidae), as it is becoming a reference cephalopod to study many facets of growth, reproduction, physiology, behavior, and systematics. This review includes topics related to species descriptions, morphology, systematics, distribution,
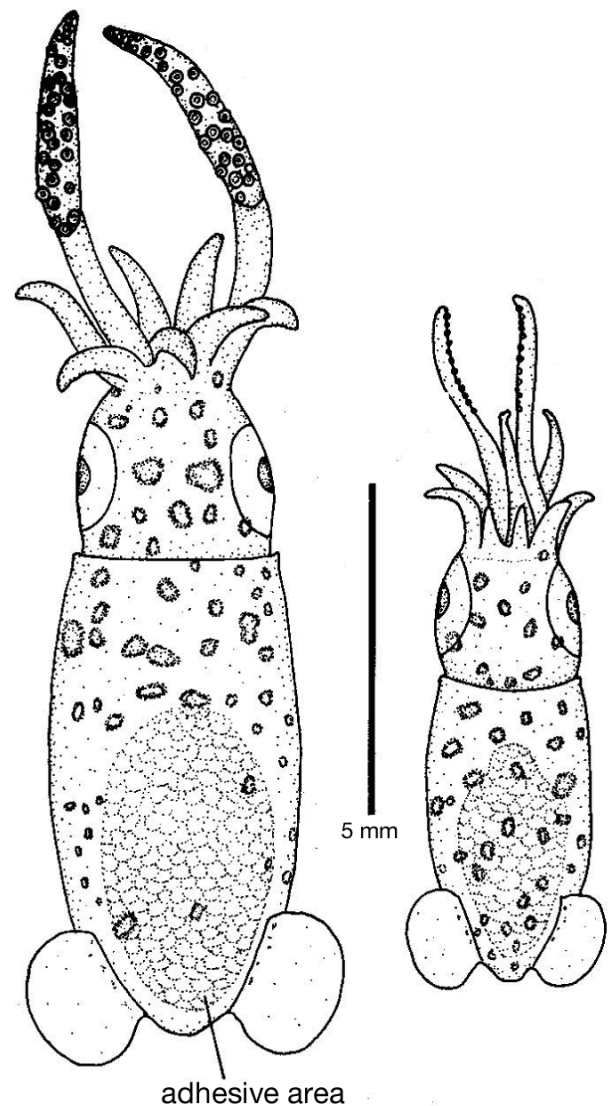

5 mm

adhesive area

Fig. 2. – Area of adhesive organ on dorsum of *Idiosepius biserialis* (from Hylleberg & Nateewathana 1991a).

behavior, reproduction and embryonic development, and growth for the Idiosepiidae.

FAMILY, GENUS, AND SPECIES DESCRIPTIONS

The first species of *Idiosepius* were described in the late 1800s to early 1900s by Steenstrup [*I. pygmaeus* from the south China Sea (Steenstrup 1881)], Ortmann [*I. paradoxus* from Japan (Ortmann 1888)], Joubin [*I. picteti* from Amboina, Indonesia (Joubin 1984)], and Berry [*I. notoides* from South Australia (Berry 1921)]. These species have many characteristics that are common within the family Idiosepiidae; including absence of an inner arm circle (*i.e.* no buccal lappets), tentacles not present at hatchling stage, anterior edge of mantle not fused with head, nuchal cartilage absent, oval attachment organ on dorsal side of body in the posterior portion of the mantle, cornea locked, short arms, with a small web between the 3rd and 4th arm, arm suckers in two rows, small size, both ventral arms hectocotylized and devoid of suckers along the length, smooth gladius, suckers of all arms enlarged in males, outer oviducts in females developed, with only one (left) functional, and the occurrence of benthic eggs (Steenstrup 1881, Nesis 1987). Only a few characters initially distinguished the earlier described species from one another. These included the number of pairs of suckers and length of ventral arms (in males), and the width of the tentacular club. For instance, in *I. pygmaeus*, 1-3 suckers exist at the base of the ventral arms in males, whereas in *I. paradoxus* there are 4-7 suckers. *Idiosepius picteti* has a much shorter right ventral arm than the left, with only one sucker at the base of each arm. Finally, *I. notoides* has 7-11 pairs of suckers along the entire length of the ventral arm (Nesis 1987).

Grimpe (Grimpe 1931) then subdivided *I. pygmaeus* into three subspecies with *I. pygmaeus hebereri* (S. Pacific Ocean), *I. pygmaeus pygmaeus* (Central Indian Ocean), and *I. pygmaeus paradoxus* (N. Pacific Ocean). Although the species distribution is wide-spread across these geographic areas, modern cephalopod researchers have not accepted these subspecies. Thirty years after this "subdivision", two new sympatric species of *Idiosepius* were described by Voss (Voss 1962) from South Africa. These included *I. biserialis*, and *I. macrocheir*, of which *I. biserialis* has longer and narrower tentacular clubs with 2 rows of suckers, with 4 suckers in the basal part of each ventral arm, whereas *I. macrocheir* has a wider tentacular club with 4 rows of suckers, and the mid-section being wider than the base. Finally, the last newly recorded species was *I. thailandicus* in 1991 (Chotiyaputta *et al.* 1991), which is characterized by only two rows of tentacle suckers and 3-4 suckers on the hectocotylized arm. *Idiosepius thailandicus* and *I. biserialis* are similar in morphology, as well as *I. pygmaeus* and *I. paradoxus*, but size differences (*I. pygmaeus* males are twice as large as

I. thailandicus males), and number of rows of club suckers are enough to distinguish the species from one another (Chotiyaputta *et al.* 1991).

There has only been one species redescription thus far on the original type species, *I. pygmaeus* (Hylleberg & Nateewathana 1991b). Several additional characters were described, including a thin gladius (Fig. 3), small narrow muscular scar located mid-dorsally above a corresponding oval depression, ventral muscle connecting the mantle to the body, and no presence of a "sinew rod" to the inner ring around the margins of the spongy substratum (loculomenta).

MORPHOLOGY AND ANATOMY

The small size, sedentary nature and relative abundance of the Idiosepiidae make them easy to collect and study. Distinct morphological differences between Idiosepiidae and other cephalopod taxa have been relatively easy to describe due to the small size and the ease of collecting animals. The first published paper on morphological characters specific to *Idiosepius* was by Adam (Adam 1986) on the buccal apparatus who compared radular and beak morphology of *I. pygmaeus* and *I. paradoxus*, and described the denticulation that is unique to this genus. Specifically, the radular structure is reminiscent to that seen in Octopods, where seriation patterns of the rachidian teeth is used for species level identification. Additionally, beaks of both species are denticulated on both upper and lower mandibles, a characteristic that is unique to the Idiosepiidae and not seen in other Decabrachian families. This characteristic is common only among octopus juveniles, establishing the Idiosepiidae as an unusual example of convergent evolution in buccal mass morphology compared to other Decabrachian families.

Morphological observations have also been "renewed" in other species that had been previously described, but not yet recorded in new geographical locations. *Idiosepius biserialis*, a species first described from South Africa (Voss 1962), was also collected from seagrass beds in southern Thailand (Hylleberg & Nateewathana 1991a). Voss had not previously described the internal anatomy of this species of *Idiosepius*, and therefore a more detailed description of the species from Thailand (Andaman Sea) was completed. Using *I. pygmaeus* as a comparison, there were key features unique to both male and female *I. biserialis* specimens that distinguished them from their sympatric species, *I. pygmaeus* (Fig. 3). Hectocotylized arms in males are much longer than arms I-III, and carry 3-7 suckers in various combinations at the base of the arm. A secondary type of cornea, which is thin, smooth, and transparent, distinguishes this species from that of *I. pygmaeus*, which has a much more opaque cornea. As in all other Idiosepiidae, there is a gladius present, which covers the posterior 2/3 of the dorsum. These main features

distinguish *I. biserialis* and *I. pygmaeus* from each other in overlapping habitats. Histology of the dorsal "adhesive organ" of the mantle was described in *I. biserialis*, *I. paradoxus* and *I. pygmaeus* (Yamamoto 1949, Byern & Klepal 2006, Cyran *et al.* 2011).

One important morphological feature in the Idiosepiidae is that their relative small size allows closer investigation of microstructural anatomy of major organ systems. One system in particular is that of the neuronal and brain arrangement in these small squids. Since the Idiosepiidae have been hypothesized to be close relatives of Teuthid squids (Carlini & Graves 1999, Bonnaud 2003, Lindgren *et al.* 2004), the importance of understanding the evolution and development of the nervous system is a key point for determining how such advanced nervous systems have evolved in molluscs. Both nervous system organization and an overall atlas of the brain in the species *I. paradoxus* have been described in detail (Shigeno & Yamamoto 2002, Yamamoto *et al.* 2003, Wollesen *et al.* 2010a, b). Comparisons to other dechabrachians such as *Loligo* and *Sepia* indicate that *I. paradoxus* specimens have a more highly developed optic tract region, with a stellate commissure not observed in *Sepia* or *Sepiola* species. This information can also be compared to recent molecular

studies, where transcriptomic and developmental data have supported the mechanisms of cephalopod camera eye evolution (Yoshida & Ogura 2011). Brain structures are very similar to other Decabrachian and Octobrachian species, indicating that developmentally the brains in most coleoid cephalopods are highly conserved (Yamamoto *et al.* 2003). Many of the finer, detailed features of the nervous system differ; apparently reflecting the comparatively inactive nekto-benthic life-history strategy of *Idiosepius* (Shigeno & Yamamoto 2002). Thus, many of the behavioral adaptations of this squid may be reflected in the patterns of organization of the anatomy of the complete nervous system.

MOLECULAR PHYLOGENY/ SYSTEMATICS

There has been much speculation on the placement of the Idiosepiidae within the Dechabrachian clade of Cephalopods. Early systematic analysis had Idiosepiidae placed sister to Teuthida (Hylleberg & Nateewathana 1991a), but more recent molecular phylogenetic analysis (both total molecules and combined analys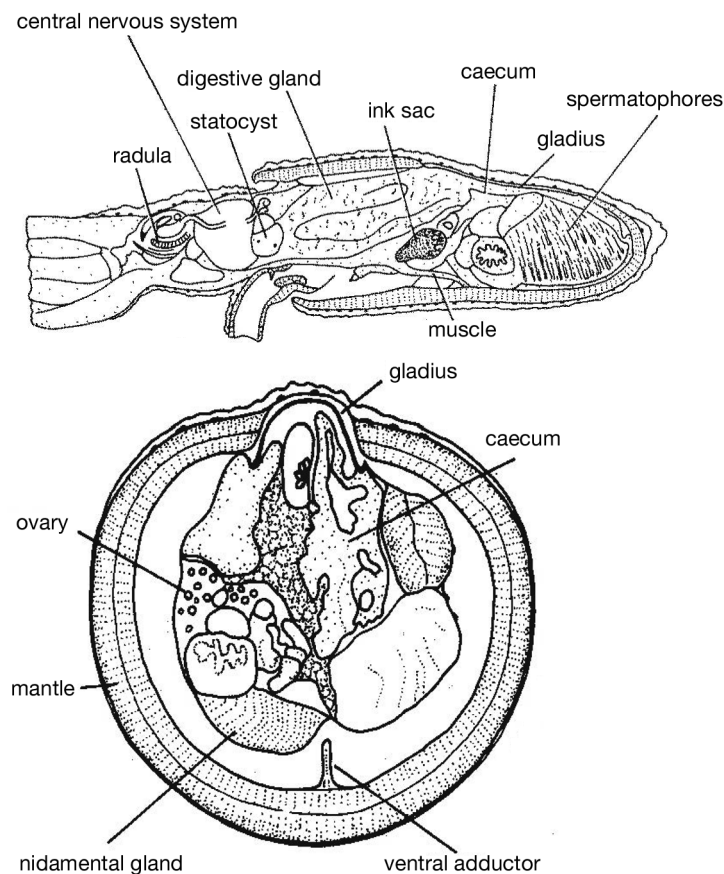is) have suggested that they are sister to Sepiidae and Sepiolidae (Bonnaud *et al.* 1996, Lindgren *et al.* 2004, Nishiguchi *et al.* 2004, Strugnell *et al.* 2006, Lindgren & Daly 2007, Strugnell & Nishiguchi 2007, Nishiguchi & Mapes 2008, Yoshida *et al.* 2010b, Lindgren *et al.* 2012). This relates to the fact that the behavior and life history strategy of the Idiosepiidae align more with other nekto-benthic squids, such as cuttlefishes and bobtail squids. Recent molecular evidence from seven species of *Idiosepius* supports the hypothesis that *I. macrocheir* and *I. thailandicus* are nested within the *I. biserialis* group, and are likely junior synonyms of *I. biserialis* (Byern *et al.* 2012). Given the genetic variability of *I. biserialis* between African and Indo-West Pacific populations and the position of *I. macrocheir* and *I. thailandicus*, the probability of introgression among *I. macrocheir* and *I. thailandicus* populations/species may explain the heterogeneity of these species groups. Additional analysis of the phylogenetic relationships have suggested that climatic conditions, rather than habitat preference or geographical isolation has led to the split between both two and four-rowed species (Byern *et al.* 2012).

Recently there has been a surge to identify the seven species of *Idiosepius* (Jereb & Roper 2005) using molecular analysis as well as morphology. Synapomorphic characters that are well defined in the family are the adhesive organ (or gland) that is restricted to

Fig. 3. – Longitudinal section of male *I. pygmaeus* (above) and cross section of posterior female body of *I. beserialis* (from Hylleberg & Nateewathana 1991a, b).

the posterior part of the dorsal mantle side and fin region (Steenstrup 1881, Sasaki 1921, 1929, Byern *et al*. 2008) (Fig. 1). Suckers tend to be arranged in either two or four rows on the tentacular club and the number of suckers on each hectocotylized ventral arm varies between species. Morphological differences between *I. macrocheir* and *I. thailandicus* did not determine any definitive species differences; both were similar to *I. biserialis* based on the 2-rowed suckers on the tentacle club (Byern & Klepal 2010, Byern *et al*. 2010). Additionally, *I. biserialis* and *I. thailandicus* do not differ in mantle length, number of suckers on the arms, or arrangement of pegs on suckers (Byern *et al*. 2010). The only molecular analysis of the genus analyzed all seven of the species of *Idiosepius*, with a number of species represented from various populations (Byern *et al*. 2010). Phylogenetic analysis using several methods recovered three major clades within the family; all individuals used at the population level were grouped together based on the analysis. Interestingly, the analysis suggests to reduce the number of species to five: *I. biserialis* (= *I. thailandicus* and *I. macrocheir*), *I. notoides*, *I. paradoxus*, *I. pygmaeus*, and *I. picteti* (Byern *et al*. 2010). Unfortunately, due to the condition of the *I. picteti* sample (formalin fixed), this sample did not provide any data for the analysis. The study did provide interesting patterns with respect to the phylogeographic arrangement of the genus. *Idiosepius biserialis* co-occurs both in an African and Indo-West Pacific clade, providing evidence of limited gene flow (both populations are morphologically indistinct). *Idiosepius biserialis* from Japan are morphologically distinct from *I. paradoxus* from the same area, yet molecular data had them grouped within the same clade (Byern *et al*. 2010). Future work should focus on the distribution, behavior, and ecology of the sympatric species, to determine if there is evidence of any gene flow or hybridization occurring between the populations.

DISTRIBUTION

Very little information regarding the location, habitat, and distribution of the Idiosepiidae has been published, except for the initial species descriptions as well as some specific records for species that have been found in different localities as previously noted. Challenges in determining the range of species distributions are associated with their small size, cryptic habit, and that when found in scientific collections are identified to genus level only. Seasonal differences in life history characteristics of *Idiosepius pygmaeus*, has been investigated in one type locality [North Queensland, Australia; (Jackson 1992)]. Unusual findings of *Idiosepius* outside of what has been considered their normal range have been recorded for the temperate *I. paradoxus* in the Russian and Japan Seas (Nesis *et al*. 2002, Sato & Munehara 2013), temperate/tropical *I. biserialis* located in Japan to Indonesia and

Mozambique (Byern *et al*. 2005, Byern *et al*. 2010), and new localities for *I. pygmaeus* in Thailand and Indonesia (Suwanmala *et al*. 2005). No population genetic studies, surveys, or records have been found for the other species of *Idiosepius* (Byern & Klepal 2007). An unusual capacity for re-introduction into habitats that do not allow for survival during cold winter water temperatures has been observed, with evidence that large scale passive dispersal is possible by adults (Sato *et al*. 2009). Adherence to drift material that can then be dispersed elsewhere might be an alternative explanation for a benthic dweller to be transported across greater geographical distances.

BEHAVIOR

Idiosepius is a prime candidate for the study of behavior given their ease at observing them in captivity, as well as studying a range of age-specific behaviors from feeding to reproduction. Earliest studies examined the adhering habit of *I. pygmaeus*, since this behavior is unknown among teuthid squids (Sasaki 1921). The morphology of the adhesive organ was described, noting that mostly columnar cells comprise the lining of the organ, in tightly packed bundles with thick cytoplasm laden with fine granules. Sasaki hypothesized that *Idiosepius* uses this organ to adhere itself to the substrate for two functions; during egg deposition and to avoid predation. Subsequently, this adhering behavior was more thoroughly investigated using *I. pygmaeus*, as well as other behaviors observed both in the wild and in captivity (Moynihan 1983a). These included diel rhythm and activity during daylight hours, solitary habits (non-gregarious), habitat preference (sea-grass and large frond macroalgae), various swimming postures (including anchoring), feeding, with notes on tentacular use, ritualized patterns (including light coloration, dark and striped patterning (camoflauge), bar shapes, and spots (dynamics), and inking.

Feeding behavior has been examined in *I. paradoxus* (Kasugai 2001, Kasugai *et al*. 2004) and *I. pygmaeus* (Roberts 1997). *Idiosepius paradoxus* has two stages of feeding upon small prey items, namely attacking and then eating (Table I). The attacking stage can be divided into three phases: attention, positioning, and then seizure as previously described in cuttlefish (Messenger 1968). Once a prey item is captured, the animal inserts the buccal mass into the exoskeleton of the prey item allowing the flesh to be ingested (Kasugai 2001, Kasugai *et al*. 2004). This behavior has also been noted in juvenile teuthid squids (loliginids, E. Vidal pers. obs.). The importance of vision in capture of prey by *I. pygmaeus* was evident when it was demonstrated that better light quality allowed greater success rate at capturing prey and reduced the time spent in the predatory sequence (Roberts 1997). Feeding behavior does not differ among individuals of various sizes or change when different sized prey items are presented.

However, *I. pygmaeus* does selectively choose particular prey items and in this study they actively selected *Acetes sibogae australis* regardless of the relative size of the squid or prey item.

Idiosepius thailandicus from Thailand was used in a comparative study with *I. pygmaeus* to identify elements of similarity as well as to identify any synapomorphic characters. In the laboratory, *I. thailandicus* displays differential sex-specific postures when adhering to eel grass (Nabhitabhata 1998). The two species also differed in their color pattern formation, prey seizure, and the larger size of females. Four types of mating behaviors were observed, including (1) males hovering above an adhering female; (2) both males and females adhering to the same substratum; (3) both males and females hovering above the substratum; and (4) hovering females above an adhering male (Nabhitabhata 1998). Males will try to mate with smaller males, but this was due to false recognition, and ceased once the larger male recognized the other as a male. Both *I. thailandicus* and *I. pygmaeus* show similarities in spawning and developmental patterns and both species fail to display gregarious or schooling behaviors.

Reproductive behavior by *I. paradoxus* has been studied in detail more than the other species of *Idiosepius* (Natsukari 1970, Kasugai 2000, Sato *et al*. 2013a). Laboratory observations of wild-caught animals have determined that there are distinct patterns of mating in *Idiosepius* (also see previous discussion under behavior). Males grasp females at the base of the arm crown and insert the right hectocotylized arm into the female's arm crown, while the left hectocotylized arm takes the spermatangia (evaginated spermatophores) from the funnel and attaches them to the female's body via the tip of the left hectocotylized arm (Kasugai 2000, Sato *et al*. 2013b). Females will mate with either one or many males in-between deposition of batches of eggs, while there is no antagonistic behavior among competing males, there is evidence of cryptic selection of males by females (Sato *et al*. 2014). Female *I. paradoxus* can remove spermatangia after copulation and will remove spermatangia of larger males and male that took longer to deposit spermatophores, appearing to actively select for smaller fast mating males (Sato *et al*. 2013a, 2014). During egg deposition, females adhere to the substrate using the adhesive organ (eel grass, macroalgae, or the side of an aquarium), with the body upside down (Kasugai 2000). Eggs are ejected one at a time, and attached to the substratum within an egg capsule (Natsukari 1970; Table I).

Cross-mating between species has been observed in *Idiosepius biserialis* and *I. thailandicus*, due to the lack of differences between the two species in their reproductive behaviors. These behaviors include both hovering and adhering positions of each sex during copulation (Nabhitabhata & Suwanamala 2008) for crossed pairs and within each species. Interestingly, both species are allopatric, with *I. biserialis* found only in seagrass beds along the

Andaman Sea coastline and *I. thailandicus* in mangroves of the eastern Gulf of Thailand. Thus, geographical isolation within the genus does not necessarily constitute major differences in reproductive behavior.

There is evidence that *Idiosepius* can display consistent inter-individual variability in their behaviors and that correlations among behaviors has allowed identification of personality traits (Horsman 2012). This study held adult *Idiosepius* sp. (undescribed eastern Australian species) in aquarium conditions and exposed them to three contexts; a threat, a feeding, and a novel environment. Fourteen behaviors, similar to those described by Sinn & Moltschaniwskyj (2005), were recorded and quantified. Correlations were evident among these behaviors and Horsman (2012) identified three personality traits; "boldness" and "responsiveness" in a threat context, and "activity" in a feeding context. In the "bold" personality trait bold individuals did not move away from the threat quickly or display color changes, while the "responsive" trait was characterized by unresponsive animals stretching the arms out towards the threat and not jetting away from the threat. The "activity" behavioral trait in the feeding context was evident by more active animals undertaking more hunting attempts and spending more time fin swimming. The significance of these behavioral traits in the success of populations is unclear as there is no correlation with body size or sex (Horsman 2012), but the correlation between physiological features and behavior was undertaken by another Honours student on the same species (Felton 2013). However, *Idiosepius* sp. demonstrated no evidence of correlations between mass specific metabolic rate and level of boldness or activity in either the threat or the feeding contexts (Felton 2013).

REPRODUCTION

Most of the research on *Idiosepius* has been on the reproductive life history strategy, since this squid has unusually high fecundity for such a small and short-lived species. Early research described the spawning mode and reproductive output of *I. pygmaeus* (Lewis & Choat 1993). A noticeable coordination between reproductive output and adult growth occurs in both wild-caught and aquarium raised *I. pygmaeus*, with females incorporating at least five times their body weight into eggs and egg coats. In addition, senescence and death is not related to exhaustion of the reproductive potential. Growth in relation to reproductive output was also investigated in *I. pygmaeus* with respect to temperature and food availability (van Camp 1997), where both factors contributed synergistically. There is no evidence that egg number, size, or batch condition decreases with successful clutches of eggs produced by a female, and no trade-offs between the size and number of eggs deposited. The capacity for simultaneous allocation of energy to growth and repro-

Table I. – Comparison of life history and behaviour of pygmy squid species. References: a, (Lewis & Choat 1993); b, (Jackson 1989, Jackson 1993); c, (Nabhitabhata *et al.* 2004); d, (Hylleberg & Nateewathana 1991a); e, (Voss 1963); f, (Jivaluk *et al.* 2003); g, (Sasaki 1929), (Kasugai 2000, 2001), (Kasugai & Ikeda 2003), (Kasugai & Segawa 2005); (Sato *et al.* 2008, Sato *et al.* 2009); h, (Natsukari 1970); i, (Nabhitabhata 1994, Nabhitabhata 1998); j, (Nabhitabhata & Suwanamala 2008); k, (Hylleberg & Nateewathana 1991b); l, (Tracey *et al.* 2003). F, female; M, male; ML, mantle length.

aspects	*Idiosepius pygmaeus*[a]	*Idiosepius paradoxus*[g]	*Idiosepius thailandicus*[i]	*Idiosepius biserialis*[i]	*Idiosepius notoides*[l]
Habitat *Food organisms* *(in captivity)*	littoral, estaury, mangrove glass and mysid shrimps (*Acetes sibogae australis*[b], *Mesopodopsis orientalis*[c])	littoral, seagrass bed amphipods (*Ampithoe* sp.), mysid shrimps (*Neomysis japonica*)	littoral, estaury, mangrove mysid shrimps (*Mesopodopsis orientalis*)	seagrass bed mysid shrimps (*Mesopodopsis orientalis*)	Seaweed, seagrass bed
Sex ratio	F > M		F > M	F > M	F > M
Size dimorphism :					
- female (average ML : mm)	F > M 17.5[d]	F > M 15, 7.8[h]	F > M 10.4	F > M 6.4[k]	F > M 20
- male	11.7[d]	12	5.9	3.6[k]	16
Mating behaviour:					
- pattern	adhering and hovering[c]	adhering and hovering	adhering and hovering	adhering and hovering	
- motion of male	no dart[c]	dart	no dart	no dart	
- position	head-to-head[c]	head-to-head	head-to-head	head-to-head	
- copulation period (s)			0.5-1	3-7	
- organ used	hectocotylus[c]	hectocotylus	tentacles	tentacles	
- region of spermatophores attachment	arms, buccal, head, mantle[e]	arms, buccal, head, neck, mantle	buccal	buccal	
Spawning behaviour:					
- pattern	adhering[c]	adhering[h]	adhering and hovering	adhering	
- substratum	rock and shell[a] aquarium wall[c]	aquarium wall and bottom[h], eelgrass blade[g]	aquarium wall, glass slide, seaweed fronds	aquarium wall, eelgrass blade	
- organ used	tentacles[a], arms[c]	tentacles[h], arms[g]	arms	arms	
- capsule attachment period (s)	30	30[h], 70-80[g]	5-10	30-50	
- batch size (no)	58.2	17-64	22.2	46.5	
- total eggs (no) / female	640[b], 100-200[c]	51.6	159.5	174.5	
- egg capsule diameter (mm)	2	1.2-1.4	1.8	1.7	
- coating layers		8-10	16-22	14-20	
- embryonic period (d)	7-14[c]	15-17	10-13	6-10	
- temperature(°C)	23-27.5	18.5-22.6	23-28	28	
Hatchling:					
- mode of living	planktonic	planktonic	planktonic	planktonic	
- size (average ML : mm)	1[b], 0.85-0.9[f]	1.16-1.22	1	1	
Life span *(statolith ring count)*	67-79[b]	140-150			91-115

(citations follow superscripts with taxa at table head, except those with details of the table text)

duction is evident as females continued to grow even during their spawning period. Although, in contrast to studies by van Camp (1997), measurements of *I. pygmaeus* statoliths to determine reproductive investment during winter versus summer months provided evidence that individuals partition a greater amount of energy in gonadal tissues over longer periods of time during winter months which slowed, but did not suspend somatic growth (Jackson 1993). Females also have greater protein concentrations, even at warmer temperatures (contrasting an earlier hypothesis that greater temperatures reduce protein concentrations in metabolically active individuals). Growth rates will slow in cooler temperatures, but accelerate during breeding (English 1981), with larger gonads in the winter season compared to the autumn counterparts (Jackson 1993, Sato *et al*. 2009).

Most research on reproductive biology has focused on the females, with limited descriptions about the *Idiosepius* male reproductive system, like most cephalopod species the males have a seminal receptacle in which the sperm are stored near the bottom of each sac. Females can then store sperm after numerous copulations, and spermatozoa can subsequently move to the seminal receptacle without being depleted after one single spawning event (Sato *et al*. 2010; Table I).

Idiosepius pygmaeus females held in captivity in 28 L glass aquaria and fed *ad libitum* on live *Acetes sibogae australis*, a sergestid shrimp, with one or two males present intermittently through the period of captivity were capable of multiple spawning events, with as many as eight egg batches produced over 22 days, and the total number of eggs produced by any one female in captivity ranged from 53-922. The number of eggs produced in a single batch ranged from 16-287, with the average number of eggs per batch 80.4 eggs (SE 8.9). Fertilization rates ranged from 77 % to 80 % and in all cases the female died within several days of the last spawning episode.

EMBRYONIC DEVELOPMENT

Developmental time of embryos is approximately 15-27 days after oviposition, depending on species (English 1981, Yamamoto 1988), in temperatures between 18-22 °C. The elliptical eggs are encapsulated by a gelatinous coat, with 8-10 spiral layers of a translucent membrane (Kasugai & Ikeda 2003). Eggs are usually 0.87-0.91 mm in length and 0.67-0.72 in width during early stages, again depending on the species (Natsukari 1970; Table I). During embryonic development, approximately 30 stages are observed (Yamamoto 1988). In *I. paradoxus*, the arm formation of the embryo follows the formula 2 > 1 > 3 > 4, with no tentacles present on hatching (Natsukari 1970). This may be related to the fact that tentacles are solely used in sexual reproduction, *i.e.*, copulation, particularly in *I. thailandicus* and *I. biseralis* (Nabhitab-

hata 1998, Nabhitabhata & Suwanamala 2008). This is not true for all other species of *Idiosepius* (see Table I).

Water temperature has an important role in all aspects of development and hatching of the tropical *I. pygmaeus*. *Idiosepius pygmaeus* fertilized eggs held in constant temperatures (20 °C, 25 °C and 30 °C) and at 12 h light/dark cycles differed in their survival; no mortalities of developing embryos occurred at 30 °C, 5 % mortality at 25 °C and at 20 °C 63-83 % of the embryos died. Embryo death at 20 °C characteristically occurred during the final stages of organogenesis. Gestation rates were also temperature dependent and were fastest at 30 °C; only 9-10 days. The slower embryonic development at 20 °C resulted in an extended gestation period of between 31-35 days. Juveniles that hatched from the surviving eggs also differed as a function of temperature; despite rapid rates of development at 30 °C, juveniles suffered greater mortality during and immediately after hatching, compared with embryos at 25 °C. Temperature also influences the number of days over which hatching occurred which was greatest at the cooler temperatures, with a single batch of eggs held at 20 °C from taking up to 17 days to hatch. Hatching time was reduced at 30 °C, with a maximum of seven days for all embryos to hatch from a single batch. As these water temperatures are typical of tropical waters, the prolonged hatching of *I. pygmaeus* at lower temperatures suggests that as development becomes more protracted the difference in developmental rates of individual eggs becomes greater. Thus, over the winter it would appear that development takes longer and hatching would occur over a longer period than during the summer.

GROWTH AND LIFESPAN

Most publications on growth and lifespan have primarily focused on tropical *I. pygmaeus*. Since *Idiosepius* are short-lived cephalopods, the ability to determine age-class and time to reproductive maturity are important features for interpreting life history changes. This is important since individuals caught in each season will be a different generation and have experienced very different environmental conditions from the generation before. Information about the tropical *I. pygmaeus* and temperate *I. notoides* (both species from Australia) allow comparisons between the two allopatric species, since they are located in different habitats. *Idiosepius pygmaeus* primarily is found in tropical waters, from southeast Asia to northern Australia; *I. notoides* in temperate waters ranging from Tasmania to the south-east coast of Australia. Statolith microstructure analysis in *I. pygmaeus* provides estimates of age at maturity (45-60 days) and growth rates in relation to maturity (Jackson 1989). Females have a much larger variation in size with respect to maturity, and estimates of growth rates and longevity of this species suggest that there are multiple generations within one year (Kasugai & Segawa

2005, Sato *et al*. 2008). In comparison, *I. notoides* statolith microstructure indicates that this species matures between 68 (for males) and 88 (for females) days, with similar sizes for the onset of maturity for both males and females (Tracey *et al*. 2003). Since this species is primarily found in colder waters, this comparative study supports observation from other marine species that temperate species live longer and grow to larger sizes in cooler temperatures, an important fact when considering the short lifespan of this family of squids (Table I).

The structure and function of organ and muscle systems has also been a targeted feature to study in *Idiosepius*, allowing the processes underpinning the rapid and continuous growth to be described. Using *I. pygmaeus*, evidence based on the ultrastructure and proximal composition of the digestive gland demonstrated a strong correlation between numbers of secretory spheres, which are esterase rich, and the time of day that the cells are being produced (Semmens *et al*. 1995). The enzymatic activity of these cells increases during times of feeding, indicating that enzyme production is very responsive to feeding, reducing the time that food is present in the digestive system, and is probably linked to the metabolic demands and physical constraints of this fast growing cephalopod. It is generally thought that cephalopod metabolism primarily uses protein or carbohydrate as an energy store; with no evidence that the digestive gland (analogous to the liver) is not used for lipid storage, but predominantly excretion in *I. pygmaeus* (Semmens *et al*. 1995). Contrary to this, ultrastructural evidence in *I. notoides* provides evidence of lipid storage in the form of large droplets of lipid-like spheres, which are contained in the caecum and digestive gland of wild-caught and captive-fed *Idiosepius* (Eyster & Van Camp 2003). These lipid stores disappeared from the digestive gland when animals were starved in the laboratory, and reappeared several days later in the caecum. Although the use of lipids is not definitive, it is suggested that *Idiosepius* uses these lipid droplets for either energy storage and/or for buoyancy control. As of this time, no further studies have been completed to determine if this is related to growth and feeding/starvation, but it is possible that the capacity for lipid storage in *Idiosepius* is species specific. Lipid storage is problematic for small individuals as internal space is limited, but *Idiosepius'* capacity to attach to the substrate reduces some of the buoyancy issues associated with lipid stores.

Along with digestion, muscle structure and function in mantle tissues has been investigated in *I. pygmaeus*, with respect to changes associated with growth, nutrition, reproduction, senescence, and captivity (Pecl & Moltschaniwskyj 1997). Using a variety of conditions, animals are plastic in respect to their growth in muscle fiber (number and size) and muscle block size. The process of growth rates are altered during captivity as a function of the relative contributions of hyperplasia and hypertrophy (Pecl & Moltschaniwskyj 1999). This reflects the fact that cephalopods in general are malleable in their allocation of energy to growth and reproduction, and are highly responsive to environmental variables. Additionally, *I. paradoxus* was used as a model to determine developmental patterns of vascular endothelial growth factors (VEGF's), which are important for inducing development and regulation of permeability of blood vessels in invertebrates (Yoshida *et al*. 2008). Interestingly, this growth factor is expressed in the blood vessels of arms and regions adjacent to the optic lobes, probably due to the formation of blood islands, a feature that is commonly found in open blood-vascular systems of other molluscs (Yoshida *et al*. 2010a).

CONCLUSIONS

The Idiosepiidae are small, yet dynamic cephalopods that represent a unique and specialized group of molluscs. Research undertaken to date provides knowledge about their distribution, behavior and reproduction, life history strategies, growth, and past evolution of the family. Yet, there are questions on the monophyly of some species pairs or groups within the genus, as well as whether all seven species are indeed "good taxa". Developmentally, the evolution of the adhesive gland is intriguing, largely because no other cephalopod species has such an apparatus for attachment and may be evolutionary related to Hoyle's organ for hatching (Cyran *et al*. 2013). The blocked tentacle development at the embryonic bud stage is unique in the decabrachia, but in fact it is a matter of heterochronic displacement. If possible, future work should focus on the rearing of the *Idiosepius* embryos to allow standardization of post-embryonic stages in life history. Difficulties in rearing newly hatched individuals past a week is because we know nothing has led to the disparate knowledge about the habitat and diet of these very small individuals. Given that these squids comprise the far-end extreme of metabolic scaling (size vs. growth) and short life cycle indeed makes them the true "mini-maximalist" of all cephalopod species.

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