The original call for participation announced the following outline:

<<The seven known species of the pygmy squid genus *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 and Chaitiamvong, 1991] are among the smallest living cephalopods. Recent studies revealed that at least some of these miniature squids are extremely short-lived, while they are “maximalists” in terms of their reproductive output.

The small adult size and the special sedentary adult mode of life of *Idiosepius* raise questions about lifetime mobility: is the post-hatching phase of planktonic life the only means of dispersal, or do adult migrations also support dispersal? How much of the biogeography is a consequence of biological and ecological constraints, and how much is due to evolutionary contingencies?

The systematics of the genus and species and the phylogenetic background of this monogeneric family may be studied under different viewpoints, using analytical approaches that range from embryological, morphological and behavioural studies, to biochemical phenetics and molecular phylogeny.

Ultimately, laboratory cultures starting out from planktonic juveniles will be necessary to study post-hatching behaviour of *Idiosepius*, and in particular to find out (1) how the tentacles, which grow out only after hatching, are functionally integrated into the arm crown for prey capture, and (2) how the dorsal attachment behaviour is established when young animals switch to the adult life style.
The aim of this workshop is to study live *Idiosepius*, to discuss their biology and the related ecological and bio-geographic aspects, and to collect all the available information on these unique cephalopods. The local coordinators will provide live animals in aquaria, so that the workshop participants may get first-hand experience in observing some of the peculiar behaviour patterns of these animals. If spawning animals are available, or eggs can be collected from the field, a hands-on session for the study of living embryos will be organized. Some emphasis will be placed on the procurement and circulation of unpublished reports on *Idiosepius*; therefore, anyone having access to such documents is kindly requested to bring at least one copy of each relevant item to the workshop. The results of this workshop are intended to be published as a group report.

Workshop Format and Group Report

During the first day of the workshop, a series of presentations and open discussions set the stage for a wider survey during the second day, with a series of parallel, in-depth reflections by subgroups, and a final presentation of the conclusions.

17 February 2003:

Jaruwat Nabhitabhata opened the session with a welcome address and a first presentation of some live animals producing egg masses in their tanks: *Idiosepius pygmaeus* and *I. biserialis*.

Sigurd Boletzky recalled the historical background and rationale of this workshop (as outlined in the introductory text) and pointed out that renewed interest in pygmy squid arose when Voss (1962) described two new species of *Idiosepius* (*I. biserialis, I. macrocheir*) – four decades after the description of *I. notoides* by Berry (1921). Voss’ work brought the number of recognized species to 6 – three decades before *I. thailandicus* was described by Chottiyaputta et al. (1991). This work, along with studies by Hylleberg and Nateewathana (1991a, b), confirmed the presence of a gladius in *Idiosepius* spp., a fact that had been previously disputed. Another important turn was the first detailed description of *Idiosepius* embryos, especially the peculiar morphogenetic retardation of the tentacle rudiments, by Natsukari (1970) – nearly two decades before complementary information and a staging system were published by Yamamoto (1988). In the same period Adam (1986) drew attention to some very peculiar aspects of the radula and beaks that were first described by Appellöf (1898). The rather slow progress in morphological and systematic studies with *Idiosepius* was finally overtaken by a much more rapid development in recent years. Biological studies increasingly drew attention to the “maximalist” performance of pygmy squid, especially with regard to fecundity (Lewis and Choat, 1993).

Jaruwat Nabhitabhata gave an overview of the biology of *I. thailandicus*. This is the smallest species of the genus and is characterized by strong sexual dimorphism, the female being 3–4 times larger than the male. Colour patterns also differ between the two sexes, the females being light brown, whereas the males are dark brown (substrate colour matching notwithstanding). Likewise, orientation in space differs in resting individuals, with females attaching themselves in a head up position, whereas males position themselves head down. During mating, males approach females, apparently using the tentacles (not the hectocotylus!) for spermatophore transfer to the buccal area of the female. Females then spread out their arm crown for acceptance of spermatophores. Mating in attached males and females may be induced by females enticing males to move down to them on the substrate. Females explore the substrate for egg attachment with arms stretched to a sharp point. Eggs are laid on seaweed using the arms. During pauses in egg laying, females undergo mating. There is no mate guarding by males.

Freshly laid eggs are surrounded by many jelly layers. Subsequent developmental events were described up to hatching after 2 weeks. As in other species, no tentacles are developed at hatching.

Main differences between the three species of *Idiosepius* found in Thailand (*I. thailandicus, I. pygmaeus, I. biserialis*) and reproductive strategies were discussed.

Finally, culture of pygmy squid was considered in comparison to cultures of loliginid
squids and sepid cuttlefish performed at temperatures up to 28°C (Nabhitabhata, 1994a,b, 1998). *I. pygmaeus* was reared from hatching, and two individuals survived to 30 days. They fed on wild copepods and mysids (*Mesopodopsis orientalis*). Adhering behaviour was not observed. One of them spent its time lying on the bottom as well as swimming. Final mantle lengths were 3.67 and 4.92 mm.

**Sean Tracey** presented a paper entitled “Life history traits of the temperate mini-maximalist *Idiosepius notoides*” (Tracey et al., 2003). Wild-caught individuals were aged by interpreting the increment structure on transverse sections of statoliths (growth rings counted at 40x magnification from natal ring to dorsal dome surface). Histological preparations established sexual maturation stages. Strong sexual dimorphism was displayed in the age at onset of maturation, females mature at 88 days and males at 69 days, with growth rates being similar in both sexes. Maximum age of around 107 days was observed in one female.

Intraspecific variability in growth rates was detected, it was proposed that this be due to the influence of SST on metabolic rate. *I. notoides* occurs from New South Wales to Tasmania, surviving temperatures as low as 10°C. In contrast, *I. pygmaeus* occurs from N. Australia to NSW and grows faster at the higher temperatures, which range from 16 to 30°C. Overlap of geographic distributions in NSW and differences between life history strategies under tropical and temperate conditions were discussed.

**Toshie Wakabayashi** presented some distribution data on *Idiosepius pygmaeus* based on plankton net catches (night samples) taken from NW Australia to S Australia. Temperatures ranged from 26 to 30°C, salinities from 34.2 to 35.2 psu. Animals were mostly found in coastal waters (0–100 m) and close to shore. Specimens were identified by tentacle and arm structures, with emphasis on early hectocotylization in males. Arm crown and beaks were studied by SEM (oral view of arm crown).

**Takashi Kasugai** summarized his poster presentation “Life cycle of the Japanese pygmy cuttlefish *Idiosepius paradoxus* (Cephalopoda: Idiosepiidae) in the *Zostera* bed at the temperate coast of central Honshu, Japan” (Kasugai and Segawa, 2003).

*I. paradoxus* is the northernmost species, occurring from Japan to S. Korea and E. China. Sexual dimorphism was pronounced, females being much larger than males. There was an alternation of generations with smaller and larger individuals correlating with warmer and cool seasons, respectively. *Zostera* beds were affected by changes in temperature, and also typhoon conditions. Temperatures ranged from 30°C (August 1998) to 6.0°C (February 1999). Mature specimens appeared throughout cool and warm seasons (both males and females). Males matured earlier than females. Sex ratio changed between seasons. If *Idiosepius* permanently resides in *Zostera* beds, it might be subject to degradation of the habitat.

Behavior in captivity: Copulation was observed not only when the female was at rest (i.e. not spawning) but also in course of laying eggs. Copulation was initiated by males. Males approached slowly, then grasped females quickly and attached spermatophores close to the female’s buccal mass. Males finding egg laying females grasped females at the base of their arms and attached spermatophores between phases of egg laying. Up to 3 males may remain in stand-by position awaiting an opportunity to copulate with one and the same female. Both ventral arms of males were hectocotylized (left arm IV has 2 small lobes at the tip; it is raised while the right arm IV, which is devoid of such lobes, is inserted into the female’s arm crown).

After copulation outside of egg-laying, spermatangia implanted in the arm crown were picked up by females (using her buccal mass, which is protruded) and might have been moved to the seminal receptacle below the mouth. On the contrary, in copulation with egg-laying females, spermatangia just implanted by males seemed to be used to fertilize spawned eggs, whereas spermatozoa in the seminal receptacle were left where they were as a reserve for subsequent egg laying. The observation of buccal mass protrusion raised the question of which parts were actually being pushed out, the buccal mass alone, or the
buccal mass along with surrounding integumental tissues. Probably the homologue of the (undifferentiated) buccal crown was also pushed out, thus forming a proboscis (NB: no differentiated buccal lappets exist in Idiosepius).

Egg laying females used all arms to attach eggs to the surface of a substrate. Egg laying lasted up to 80 seconds for a single egg. Uninterrupted terminal spawning continued during more than two weeks. During egg laying the oviducal jelly surrounded the chorion of each egg and was immediately covered by nidamental jelly, which subsequently “fused” with the added nidamental jelly of the next following eggs.

Rearing of juveniles: juveniles preferred mysids over Artemia. The empty exoskeleton of the prey was discarded after removal of the flesh by the protruded buccal mass. Only 3 individuals were raised to the age of 19 days, 1 individual reached the age of 26 days. At hatching, there were no tentacles. After 2 weeks, individuals began to adhere to a substrate using the mantle tip (remainder of Hoyle’s organ?). After 3 weeks, the tentacles were visible within the arm crown. The oldest specimen (26 days) had a mantle length of 3 mm, the tentacles had a length of about 2/3 arm length. At that stage, the short tentacles were probably used like arms (fast musculature developing?).

Mary Lucero gave an informal presentation of the cephalopod olfactory organ and its physiology, recalling the definitions of taste (contact chemoreception in suckers, lips, fins, around eyes) which involves solitary chemoreceptor neurons and smell or olfaction (distance chemoreception in olfactory pit/olfactory organ, situated ventral and posterior to the eye). Benthic organisms have better chemosensory systems than pelagic organisms. Solitary organisms use distance chemoreception. Therefore, since Idiosepius is both benthic and solitary, it should have a better chemosensory system than other types of squid.

Behaviorally relevant odors for cephalopods were identified from the use of bait with amino acids (bait accepted) or bait with betaine (bait rejected) (Lee et al., 1994). Additional behavioral studies which mapped the chemosensory regions of the squid showed that the region of highest chemical sensitivity was the olfactory organ (Gilly and Lucero, 1992).

The cephalopod olfactory organ is composed of sensory neurons and ciliated support cells that generate a whirlpool to pull the water towards the receptor cells. Neurons within the olfactory organ send fibers to the olfactory lobe of the brain and to the optic gland (supposed importance for gonadal maturation). Its anatomy suggests both olfactory function and pheromonal function (possibly triggering egg laying). Cross sections of squid olfactory organs exhibit an epithelium with pseudo-stratified layers of support cells and five different morphological subtypes of olfactory neurons (Emery, 1975).

Electrophysiological recordings from squid olfactory neurons showed that odors activate either excitatory or inhibitory receptor potentials.

It will be interesting to look at different morphotypes of cephalopod olfactory neurons and map onto them the functional responses to different chemicals.

Shuichi Shigeno presented his observations on nervous system development in Idiosepius in relation to behavioural changes. An SEM study of embryonic development illustrated some peculiar features, such as the presence of so-called pseudoarms (ostensible duplication of arm rudiment) in the early stage of brachial morphogenesis, or tuft type cilia in the mantle cavity, similar to those described in sepiolids. Toothed beaks were differentiated in the hatchlings. Cup-shape of the olfactory organ was unique to Idiosepius, also its morphogenetic “migration” to the definitive ventral posterior position in relation to the eye. Other peculiarities existed in Hoyle’s organ, in the lateral line homologue and in ciliary distribution in general.

An atlas of the embryonic brain in Idiosepius paradoxus is now available (Yamamoto et al., 2003). A description of the neural network formation using confocal microscopy was given. Comprehensive gene cloning from the Idiosepius brain was performed to recognize brain elaboration processes. Expression of neuron specific glycoprotein mRNA is now being studied.

Adult Idiosepius had disruptive chromatophore patterns (similar to young loliginids). Is this a
Idiosepius: ecology, biology and biogeography of a mini-maximalist

synapomorphy suggesting a monophyletic group uniting pygmy squids with loliginids, or is the brain structure/anatomy more closely similar to the Sepiolidae?

Overall, in the phylogenetic context, where does Idiosepius have its place? Does dwarfism play an important role in the processes of development, life history strategy, and reproduction? Reduction of tentacles perhaps is less important than a similarity to ommastrephid squid brain development.

Mike Steer and Jennifer Mather led an open discussion of behavior, from aquarium studies to the questions of natural in situ behaviour of Idiosepius compared to other cephalopods, focusing on questions of species’ ecology and possible implications for biogeography. Emphasis was placed on the links between ecology, neurophysiology, and physiology when behavior was analyzed. The ultimate requirements were that an individual must eat, avoid predation, and reproduce. The following questions asked how this is achieved by the animals.

Foraging: possible options were sit and wait, or actively hunt. Related questions were: if they sit and wait, for how long? At what time of the day do they hunt? What range of species do they take? What cues are used for prey capture? Do they return home after hunting?

Predator avoidance: possibilities included passive camouflage, a sequence of strategy changes, and deception by inking.

Reproduction: how does an individual of a sessile species find a mate? Males are supposed to play the first active part. Chemical cues could be involved in mate finding.

But females may be selective as well. Who initiates, who ends mating? How does a female choose a substrate for egg laying? How many eggs are laid at one site?

Habitat selection: to what extent is juvenile settlement selective? If there is selectivity, what are the basic cues?

Activity cycles: are the settled individuals permanently adhering to their chosen substrate, or only during a certain time of day? In other words, are there well-defined periods of activity during a 24 hour cycle?

Ecological implications of Idiosepius behavior led to addressing growth in relation to food availability (crustaceans being the main prey), perhaps involving chemo-reception under dim light conditions. Possible alternative modes of “grazing” (Moynihan, 1983) may result in various requirements for different growth stages. Growth also needs to be viewed with regard to sexual dimorphism in adult size and related effects on social interactions before and during reproduction.

The general poor knowledge about biogeography of pygmy squid is due to the virtual absence of empirical data on natural dispersal, in terms of juvenile and adult displacement (including rafting), but also regarding eggs that might be attached to drift weed. Aquarium studies can address these questions, but will provide much less than field studies.

Jutamas Jivaluk summarized her poster presentation “Description of hatchling of Thai pygmy squid, Idiosepius thailandicus Chotiyaputta, Okutani and Chaitiamvong, 1991” (Jivaluk et al., 2003). The mantle of hatchlings was firm and cylindrical, bell-shaped with round blunt end. Fins were separated, small, sub-terminal, oval in outline. Dorsal mantle length was 0.85-0.90 mm.

The head was nearly square in outline, with large eyes situated in the anterior corners. The arms were short and stout with blunt tips, suckers were in 2 rows on arms II and III. Arm formula: II, III, I, IV. There were no tentacles.

Up to 30 chromatophores covered the dorsal side of the mantle, 15-20 the ventral side. Arms had 1-2 chromatophores on the aboral side.

All these characters allowed differentiation between hatchlings of I. thailandicus and those of I. pygmaeus and I. paradoxus.

18 February 2003

The essential insights gained during the first day were briefly surveyed. Some open questions were: How many species are there really in the genus Idiosepius? (cf. English, 1981). How are they related to each other? Are I. thailandicus and I. biseralis sister taxa? In mature females, what is the function of the right oviducal gland, how much does it contribute to the entire jelly mass during spawning? Where is the lower limit of temperature tolerance in different species of Idiosepius?
Laure Bonnaud and Michele Nishiguchi then led an open discussion of the placement of *Idiosepius* in the system of coleoid cephalopods. The following questions were approached: What are the phylogenetic hypotheses of the group? What are the important sister groups to the Idiosepiidae? What are the phylogenetic relationships between different species? There was general agreement that formalization of relationships must be sought by using cladistic analysis in the sense of phylogenetic systematics.

This led to the following questions: What are likely plesiomorphic characters (widely common ancestral characters) and what are likely apomorphic characters (common derived characters in species that share a more recent common ancestor)? How do we as scientists first obtain a “phylogenetic framework” to then address more comparative questions regarding ecology/physiology/biogeography? Can similar patterns (morphological, physiological, ecological, behavioral) be mapped onto the phylogenetic framework to give us clues as to the relationships, evolution and radiation of this unique group of cephalopods? What characters are likely homoplasic (convergent characters) that might obscure the phylogenetic relationships between *Idiosepius* and other Decabrachia? It is advisable to preface an investigation with a phylogenetic framework to generate and orientate questions regarding the physiological, morphological and biochemical aspects that may further improve the entire analysis. An essential question in this type of analysis is: What is a good character? Can molecular and morphological characters provide « mutual elucidation »?

Molecular data are not as static as morphological data. It is harder to resolve these characters for analyses because the ancestral state of the nucleotide is unknown. How many transitions/transversions/gaps occur, what type of cost can one assign each one?

Phenetic methods cannot be expected to refine a phylogenetic hypothesis.

**In-depth reflections by sub-groups**

The following items are fair copies of the handwritten notes collected at the end of the session.

**I. - Behaviour**

- Why study behaviour? Necessary to know if the species considered is a model species useful for form-function relationships needed for keeping in the laboratory.
- Why *Idiosepius*? We need to understand the extreme to better know the usual (extreme in size, attachment and sedentary lifestyle)

**Habitat selection.** See adhesion as an energy conservation strategy needed because of the small size.

Why? Refuging, similar to the octopus den, notice also there is ‘opposite’ countershading, camouflage patterns.

Adhesion is not selective – any surface will do.

**Social organization.** What male/female proportion, probably near 50:50.

Are they clumped? Solitary? Distribution may be dictated by shelter availability – distribution has not yet been studied.

**Reproduction.** Note the high fecundity of females, eggs laid in batches over time – necessity here for energy maximization to manage to produce all those eggs.

- Mating strategies – how do sperm get selected: active selection, selection by the female, sperm interaction, sperm removal.
- Initiative can be both by male and female – there are a lot of males mating, and they may select ‘targets’ by size.
- Recognition by female postures, size, would there be chemical cues?
- There are color patterns – males can be dark brown, dark/pale halves, side stripe, also flashing ‘corner’.

**Feeding.** The animals can forage by ambush, out and catch, maybe even ‘grazing’, the usual cephalopod variety.

Back to our energy conservation theme –> any way that works.

Why can’t cephalopods be smaller than *Idiosepius* – and what do you do to be good at being small?

**Predator Avoidance.** Note hiding and camouflage, inking as a ‘last resort’, can be a ‘mimic’ ink blob.
Notice the habitat is close to but never on the bottom.

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Keeping *Idiosepius*:
They tolerate a wide range of salinity and a reasonable O2 level, temperature range is wide but species-specific, *I. paradoxus* 10°–25°, *I. thailandicus* 22°–32°.

Food: they eat crusateans, a variety is fine, size isn’t a big problem and *I. paradoxus* doesn’t eat fish.
They need an attachment surface but it doesn’t have to be *Zostera*.
Light? Maybe dim – but undersurfaces are dim anyway.
Very small *Idiosepius* are a problem for food – NOT *Artemia* or crab zoeae, maybe newly hatched mysids and zooplankton.
If they live in the plankton, we should go collect food species there.

2. - Morphology and Physiology

**Morphology**

(A) Hatching
- (1) Chromatophore patterns? Only smaller numbers related to hatching size? *I. thailandicus* < *I. pygmaeus*?
- (2) Smaller suckers? Hatchling size *I. thailandicus* < *I. pygmaeus*
- (3) The brain is very immature but all lobes are differentiated (check olfactory lobe?)

(B) Development
- egg size 1 mm or smaller (laid in layer, unlike e.g. ommastrephid spawn)
- embryonic period very short
- pseudo-arms, as are typical in sepioïds and sepoliids
- beak structure: toothed beaks
- cilia: no smooth type cilia
- heterochronic retardation of tentacles (anlage appears in the embryo): 26 days after hatching tentacles become similar in length to arms
- cup-shaped olfactory organ – relation to benthic life?
- jelly morphology: nidamental jelly fusing into one coat
- gland-like organ at the lip (base of arm crown)
- hatching lives as paralarva (at least up to the age of 16 days)

**Physiology**

Very few physiological studies have been performed in *Idiosepius* spp.
A mostly anatomical study described the digestive system of *Idiosepius*. Unlike most cephalopods, *Idiosepius* utilizes external digestion. Several unique morphological characteristics are associated with external digestion including an “s-shaped” esophagus, a ciliated stomach lining instead of the usual chitinous lining, and absorptive capabilities in the intestine and caecum. There was no evidence for energy storage in the form of glycogen or lipid in digestive gland cells. In general, the digestive system of *Idiosepius* shows similarities to loliginid species, especially paralarval forms, and differs from *Sepia*.

3. - Systematics

Observations of selected specimens under a dissecting microscope showed the following characteristics:

*I. biserialis* from Phuket
- rounded body end
- olfactory organ near eye
- pale in fix – no chromatophores
- clear cornea in fix
- back fins attached oblique to body
- rectangular adhesion organ

*I. biserialis* from South Africa
- pointed body end
- olfactory organ near eye
- pale in fix – many small chromatophores
- opaque cornea in fix
- back fins attached vertical to body
- oval adhesion organ

*I. thailandicus*
- pointed body end
- olfactory organ farther from eye
- dark in fix – many small chromatophores
- clear cornea in fix
- back fins attached vertical to body
- oval adhesion organ
4. **- Phylogeny**

   It was discussed that both an internal phylogeny for all 7 species of *Idiosepius* be pursued in order to better understand sister taxa relationships, particularly for those species which have habitat/niche overlap. In particular, *I. biserialis* from both S. Africa and Thailand should be investigated thoroughly in order to establish if they are indeed the same species (the same holds for *I. pygmaeus* from Australia and Thailand), or are separate subspecies/populations. Also, the overlap between *I. pygmaeus* and *I. notoides*. This can be accomplished quite easily with a molecular phylogenetic analysis combined with morphological data from each of the species and their respective populations.

   As for the position of *Idiosepius* within the Decabrachia, it was determined that more representative taxa for related "in-groups" and "out-groups" were needed to ensure the placement of *Idiosepius* with the squids. Although some preliminary evidence suggests that the family Idiosepiidae is sister to the Loliginidae, more data are needed to support their placement and to also answer the question of which families are sister to this unique group of squids. According to earlier data by Bonnoud *et al.* (1996, 1997, 2002), it appears that other squid families than Loliginidae, such as the oegopsid squid families Ommastrephidae and Enoploteuthidae, are possible candidates for sister taxa to the Idiosepiidae. There was general agreement that removal of the Idiosepiidae from the order Sepiolida was appropriate for placement of this family.

**CONCLUSION**

   The survey of established knowledge and open questions relating to *Idiosepius* as achieved during the workshop convinced all the participants and other people interested in the subject (see Pecl, 1994; Roberts, 1997; Semmens, 1993; van Camp, 1997; Pecl and Molschanewskyj, 2003) that the time was ripe for a synthesis. Therefore it was decided to assemble available data, especially from unpublished dissertations, in a monograph of the genus *Idiosepius*, which should be published before the CIAC2006 symposium.

**REFERENCES**

   [with translation of title where appropriate, and other indications]


