5. RESULTS

5.1 Taxonomy

The following work has been carried from 1985 to the present. When the author began his research no biologist was studying the taxonomy of this area and work had to start from the beginning. In the later years the author has collaborated with Dr Jacquie Rifkin on some of the taxonomy of species mentioned here, some of which was published in “Venomous and Poisonous Marine Animals: a Medical and Biological Handbook” (Rifkin, in Williamson *et al* 1996 p.147-155). However, the author has researched the following work independently of any other worker.

5.1.1 Carybdeid taxonomy

Morphological studies of Morbakka

The first opportunity for study presented in 1985 (Fenner *et al* 1985) after two fresh specimens were caught in the Mackay Harbour, north Queensland. Over the next 10 years further live specimens caught in the north Queensland area by the author or his practical researchers (see Appendix C) were also examined.

Preserved specimens were also examined from the Smithsonian Institute, Washington, USA, from the British Museum of Natural History, London, and those from a collection of Loisette Marsh of the Western Australian Museum, Perth.

Morbakka - a large carybdeid

Two large carybdeid specimens were found in Mackay Harbour by the author in April 1985. The larger specimen was 130 mm in diameter across the body of the bell; the height of the bell was 180 mm. The other specimen was 120 mm in diameter, and had a bell height of 150mm. Each specimen had four large mauve tentacles; one attached to each corner of the bell. The tentacles were 600 mm when extended, although they contracted to a length of 200 mm. After preservation in 10% formalin and seawater they contracted even further to just 60 mm. They were ribbon-shaped, 10 mm wide, 3 mm thick and had a multiple transverse bar pattern similar to that of *Chironex fleckeri.* The centre canal in the pedalium did not have the "hook" or "rose-thorn" appearance. The bell was covered with numerous warty mauve mamillations containing hundreds of nematocysts (stinging cells)

which were capable of causing even the thick skin of the palm to "tingle" when the animal was picked up.

These carybdeids were similar to *Tamoya haplonema*, but did not comply precisely with the description for *T. haplonema* Müller 1859 (Kramp 1961). For this reason Southcott suggested the name "Morbakka" should be used for the jellyfish until the taxonomy was clarified (Southcott 1985). The name was derived from "Moreton Bay carybdeid medusa" as many specimens had been caught or described, and a number of envenomations reported from this enclosed bay near Brisbane, Queensland. However, specimens have been seen and reported regularly in waters from Port Douglas in the north to Moreton Bay in the south, and even on sporadic occasions as far south as Sydney in the south (Fenner 1991; Fenner *et al* 1995), including a fairly major envenomation on a tourist in November 1996 (Case history in 5.6.3).

The specimens agreed with most of the major characteristics described for *Tamoya haplonema* Müller 1859 (Kramp 1961) - except that they had no gastric cirri on the stomach walls, or elsewhere in the medusa. Uchida had described the loss of gastric cirri as a normal developmental phenomenon in larger *Tamoya* in his earlier work (Uchida 1929), but then ignored this comment in his later paper (Uchida 1970), stating that gastric cirri were “present” in *T. haplonema*. Southcott reported a previously undescribed large carybdeid, that lacked gastric cirri, and was probably identical to these, but “its taxonomy has never been resolved" (Southcott 1967).

Species identification

In 1990 the author suggested that there were 2 species (Fenner 1991). Type 1 was up to 8-cm diameter, 6 cm high with tentacles 10-50 cm long, and tended to occur in waters south of Mackay. Type 2 was much larger, with a different shape with a bell up to 14 cm diameter and 18 cm high. The tentacles may be 1 m long and 1 cm wide (Fenner *et al* 1985) and occurred in waters from Mackay north.

Before 2 types were discerned, Southcott (1985) had suggested the name 'Morbakka' (derived from 'Moreton Bay carybdeid'). The name refers only to Type 1 specimens, as these alone have been collected in Moreton Bay, and off southern Queensland and northern New South Wales, whereas Type 2 specimens have been found only off Queensland north of Mackay (21' 9' S). The author is still examining large carybdeids from Darwin and Western Australia.

A study of Type 1 by Simon Moore at The British Museum of Natural History (London) in 1984 suggested that it was *Tamoya virulenta* Kishinouye 1910, but suggested that examination of the type species from Japan was really necessary for confirmation. Type 2, although generally resembling *Tamoya haplonema* shows only minor differences, including the lack of gastric cirri (Fenner 1991).

More recently, work by Rifkin suggested that the species present in north Queensland (from at least Mackay, northwards) is sufficiently close to the original description to be addressed as a sub-species, which she called *Tamoya haplonema fenneri* (J Rifkin, 1996, personal communication). She also agrees that the specimens caught in Moreton Bay, which are smaller than those caught in Mackay Harbour, match the description by Kishinouye (1910) of *Tamoya virulenta.*

Laboratory investigations of skin lesions

To permit detailed investigation of the effects of the "Morbakka" sting under controlled conditions, envenomation from the specimen caught in Mackay Harbour was undertaken in a laboratory setting.

A mark was made on the forearm of the author, who had no history of allergy, and a photograph taken of the normal appearance of the skin in this area. The subject had been exposed to the sting of a *Chironex fleckeri* on several occasions in previous years in research projects, with no allergic responses.

A piece of tentacle 20-mm long by 10 mm wide, with a thickness of 3 mm, was placed on the inner aspect of the forearm where there were very few hairs. The sting was not painful but felt distinctly "prickly". The tentacle piece was left on for three minutes, occasionally being pressed on to the skin to facilitate the discharge of nematocysts. The tentacle piece was then removed and a skin scraping taken by scraping a razor blade over the skin several times at a 90 degree angle. The scrapings were placed on a glass slide and examined microscopically for nematocysts. The tentacle was embedded in wax and also examined microscopically.

Results

When the tentacle piece was removed it left small, red, raised wheal with the same dimensions. In a band 20 mm around this wheal a marked "capillary flare" or erythema was present. Over the next 30 minutes the central wheal remained raised, but the colour faded until it became white. The surrounding erythema

seemed to become more intense in colour and numerous "punctate" lesions of less than 1 mm in diameter formed up to 10 mm around. It looked as if the skin was burning and so perhaps the name "fire jelly" is apt! A punch biopsy was taken at this stage from the edge of the envenomated area.

Over the next two hours the central area where the tentacle had been in contact with the skin became red again and remained slightly redder than the surrounding erythema. A second punch biopsy was taken after two hours. There had been no systemic effects during this time.

The envenomated area remained red and itchy for three days before the itch became less troublesome; the skin remained raised and red for two weeks and so a third biopsy was taken at 14 days. The redness slowly faded during the third week until nothing was visible a month after the original envenomation.

Histological examination of the punch biopsy of the skin taken at 30 minutes after envenomation showed oedema of the papillary dermis with dilatation of the superficial vessels only. However, in the second biopsy taken 90 minutes later, small numbers of eosinophils, some degranulated, were present around the vessels. In this later specimen, occasional thread tubes (which connect the nematocyst to the barb) could be seen penetrating the epidermis and dermis as far as the superficial vessels. At the site of penetration the squamous cells had undergone eosinophilic necrosis and, in the dermis, collagen fibres around the thread tubes appeared to be disintegrating.

The final biopsy 14 days after the original laboratory envenomation showed a superficial and deep perivascular lymphohistiocytic infiltrate which contained small numbers of eosinophils and was similar to the reaction seen in coral dermatitis and some arthropod bites.'

Carybdea spp. (California)

Recently, through the `Cnidaria' closed network on Internet, the author has reliable information that a carybdeid occurs throughout the summer amongst sea weeds at a depth of about 5-15m. Specimens are caught regularly by Shane Anderson (1997, personal communication) for the University of California and the Monterey aquarium, where they are put on display to the public. To date none have lived more than a few weeks.

Anderson describes the maximum bell size he has caught as 4cm high and 3.2cm wide, with the smallest just 3cm bell height. The tentacles when retracted are just 4cm long, but may extend out to approximately 150cm. The bell is covered with smallish (approximately 1mm) `flecks', giving it a peppered appearance. No stings have been reported, even though these jellyfish are present near the main surfing beaches of California. As happens in Australian beaches, perhaps swimmers and surfers are stung, although not too severely, and so are not reported.

A specimen was obtained from Anderson and examined by the author. There were no mamillations on the bell which was 2.5cm diameter. The sensory niches (rhopalia) had the classical `hare-lip' appearance of *Carybdea* spp. (Fenner, in Williamson *et al* 1996, p 239)(although Kramp [1961, p 305] describes *C alata* as having `normal' lips around the rhopalar niche). Sheets of gonads extend down the sides of the bell suggesting the specimen is reasonably mature, and certainly having reached adult state, if not maximum size. The gastric cirri extend horizontally from the stomach and are not dendritic (branching) but they are arranged in rows. Unfortunately, on the specimen received, no velar canals could be distinguished (see below).

The author felt the carybdeid did not fit any of the characteristics listed by Kramp 1961 for *Carybdea alata*, *C. marsupialis*, *C. rastoni*, *C. sivickisi or C. stiasnyi*, nor any *Tamoya* spp (see section 5.1.1). The closest was *C. rastoni* which is supposed to occur all round the Pacific. *C. rastoni* from South Australia previously examined by the author had two branched velar canals in each octant and the gastric cirri originate in multiple locations, not like tree trunks.

This caused a lot of discussion on the Cnidaria net. Spencer (Satterlie & Spencer 1979) suggested it was *Carybdea rastoni* although Arnesson (Larson and Arnesson 1990) had later identified it as *C. marsupialis*.

Larson and Arnesson (1990) based their identification on the works of Bigelow (1938), who stated that dendritic (branched) gastric cirri were present in each corner. They studied 13 specimens from La Jolla and Santa Barbara in California and determined that all were *Carybdea marsupialis* and that all previous records of *C. rastoni* from southern California were probably *C. marsupialis*. They reported "clusters of gastric cirri at the 4 corners of the stomach, each arising from a single trunk (or rarely from 2)" - but did not mention velar canals. They also stated the distribution to be the tropical and subtropical Atlantic and Mediterranean; in California off Santa Barbara and infrequently off La Jolla; possibly further south in Mexico and

central America. It seems both authors were familiar with *C. marsupialis* as they had spent time in Puerto Rico working on medusae during their masters degrees, and had also studied specimens *of C. rastoni* from Australia and of *C. marsupialis* and the Bahamas (Larson & Arnesson 1990).

Mills (C Mills 1997, personal communication) suggested that the species from California, which may be *C. marsupialis* is supposed to be larger than *C. rastoni.* Matsumoto (1997, personal communication) states that a *Carybdea* spp. from Hawaii, which he thought may be *C. rastoni*, has two branched velar canals in each octant, but with gastric cirri arising from one or two large trunks. Otherwise he described it as being the same size and with all the other characteristics of *C. rastoni* from South Australia, which he has studied in some detail.

With all this confusion, as has been met before with other cubozoans, the author feels that the whole subject needs further consideration with the characteristics and species redefined as being the only way to clear this confusion, which occurs with so many species, especially cubozoans. He also agrees with Matsumoto (1997, personal communication) that "Biologists may have to make a decision as to whether the velar canals or the gastric cirri are the more important. Either that or perhaps assessment of their DNA may be the only way to solve the whole problem of carybdeid (author - and chirodropid?) taxonomy". Then, complicating matters even further, he also mentioned that "J Crowe from the Waikkiki Aquarium (Hawaii) may have at least one, and possibly two new species."

5.1.2 Chirodropid taxonomy

Until recently species names have not been allocated to chirodropid specimens worldwide with any certainty. Even now, the distributional and epidemiological data is thin, as jellyfish causing fatal or serious human envenomation are rarely caught at that time. Unless this accurate data is recorded it is not possible to attribute deaths to specific jellyfish species. Even research into the venom and possible antivenom cannot be confidently started until this question is settled. The author, through travel and personal contacts, has collected many specimens of chirodropids from around the world (see below) and was collaborating with a biologist (Dr J Rifkin 1990-1995), to help bring more knowledge to the area. Much of the research centres around the variation present amongst chirodropid specimens in general as even the degree of maturity of the jellyfish affects its appearance, thus making accurate identification of the species difficult. It has only been in the last few years that a more accurate

classification of the world chirodropids has been suggested (Rifkin 1996 in Williamson *et al* 1996, pp150-155).

This complex subject is still being pieced together. In the identification of the chirodropid species causing envenomation, or even death, the specimen has to be caught at the time. Specimens often have very similar characteristics, although with its strange alignment of tentacles arising from the pedalium, along with its very long superior gonads and long upwards extension to the pedalial canal, *Chiropsoides buitendijki* is probably the easiest chirodropid to identify. However *Chironex fleckeri* in its usual habitat of north Australia is also easily differentiated from the Australian-named *Chiropsalmus quadrigatus*, although this latter species is named incorrectly. Table 1 below shows some of the differences between species. However, apart from the obvious characteristics shown below, it is very difficult at times, especially when the specimen is damaged (eg. whilst being caught, particularly in a net when tentacles become easily torn off) to precisely identify the species.

Figure 6 – Chirodropid anatomy

The Smithsonian Institution

In 1990 I visited the Smithsonian Institute, Washing ton, USA, to examine Mayer's chirodropid collection in detail. I was able to confirm Barnes' comments (Kinsey 1986) that at least two of the chirodropids in his collection that he caught in the Philippines in 1908 (Mayer 1910) were *Chironex fleckeri*, despite the fact that this genus and species was not described until 1956 (Southcott 1956). All six

specimens were labelled *Chiropsalmus quadrigatus*. Two were morphologically different to *Chironex* and are perhaps the true *Chiropsalmus quadrigatus*, and two were not in good condition and I was unable to make an accurate identification.

I also took the opportunity to study the museum's *Chiropsalmus quadrumanus* specimens as well as their entire collection of cubozoans, as *C. quadrumanus* has been described within the tropics of the eastern border of the Americas, including Puerto Rico (Cutress, in Williamson *et al* 1996, p260). I had previously examined similar specimens of *Chiropsalmus quadrumanus* from Puerto Rico. These had been supplied to me by Bertha Cutress, a researcher in the University in Puerto Rico (now retired), wife and co-researcher of the late Professor `Chuck' Cutress who did so much work on the life cycle of *Carybdea alata* in the Caribbean.

A brief summary of my findings from these studies is listed below (Table 1).

In the same trip I inspected specimens of cubozoans in the large collection at the British Museum of Natural History. Similar to the Smithsonian Institution, there were specimens that were incorrectly labeled, including a specimen that I would have identified as *Chiropsalmus quadrigatus.* It had been caught in the Philippines in 1947 and has the characteristics of *C. quadrigatus* (see below), but was labeled as *Chironex fleckeri*, despite being prior to the date that Southcott (1956) had described the new species and genus. Although it is probable that specimens of *Chironex fleckeri* could have been caught before they were formally identified, some specimens, as above, seem to be incorrectly labelled, confirming the poor classification and identification of cubozoan specimens world-wide.

Fortunately for a successful medical management there seems to be little need to specifically identify a species (or even a genus), rather than just a general identification as a chirodropid – "a box jellyfish with multiple tentacles arising from each corner." In the work of the author and his colleagues the effects of envenomation, be it mild or serious, are very similar. First aid and medical treatments can then be based on the signs and symptoms produced, which are also very similar. For first aid and medical treatment see section 5.8.

<u>Chiropsalmus quadrumanus</u>

Distribution

The eastern coast of the Americas between 10^{o}S to 34^{o}N, including Puerto Rico in the Caribbean (Kramp 1961).

Appearance

(Smithsonian specimens)

Most of the features were distorted by the preservative. The gastric diverticula were shrunken and distorted and the tentacles twisted, making assessment of their arrangement impossible. Distinguishable features were: -

- Finger-like superior gonads
- lateral sheet-like gonads
- complex pedalia, with a volcano shape similar to C. quadrigatus but without the sharply up-pointing `rose thorn' corniliculum.

Chirodropus gorilla

Distribution

Reported from Liberia, 7^{o} north of the Equator (Kramp 1955), to South Africa, some 30^{o} south (Stiasny 1931). It is possible this distribution is under-reported in the northern distribution, as most chirodropid species appear to extend some 20^{o} or so either side of the Equator (Fenner, in Williamson *et al* 1996, p268).

An expedition from Spain recently caught several specimens in the Benguela Current off Angola, West Africa, 23^{o} south of the Equator (Pagès *et al* 1992). A specimen from the expedition was kindly donated by one of its members, Professor J-M. Gili of the Marine Sciences Centre in Barcelona, Spain to the author. This specimen and another specimen were studied when the author visited the British Museum of Natural History.* It is described below, and comparison made to other chirodropids in Table 1.

* Apart from the specimen in the British Museum and one in Leijen Museum, Professor Gili has the only other specimens in the world that are in good physical shape. These he caught from his expedition, and kindly donated one to the author for his personal collection.

Appearance

Chirodropus is very similar to the other chirodropids. The bell is up to 130 mm height, slightly less in diameter, with between 9--11 tentacles per pedalium (36-44 total). Specimens examined by the author found the gonads attached to the subumbrella sides of the interradial septa and having grape-like clusters at their free edge (ie they were fairly mature specimens) – this was as Mayer (1910) and

Kramp (1961) described. The pedalial canals had a characteristic `rose-thorn' seen in only one other chirodropid (*Chironex fleckeri*), although several carybdeids.

Chiropsalmus quadrigatus – Okinawa

I had the opportunity to inspect 5 specimens of this species whilst in Okinawa. They were immature, 3-5cm bell diameter with 6-7 tentacles per pedalium. The gonads were not developed but appeared similar to *Chironex* at a similar stage. The pedalial canal has an acute `volcano-like' angulation, not quite the rose-thorn shape of *Chironex.*

Dr Araki and Cheryl Lewis have been studying this animal in the past few years. I am indebted to Cheryl Lewis for her much fuller description in 5.7.3 below.

Chiropsoides buitendijke

In September 1995 I visited Dr Malik Fernando in Sri Lanka. The previous year Malik had caught some chirodropids whilst Scuba diving and had preserved them in formalin saline. Examination of these specimens showed they were different to anything I had seen before although they matched the description in Kramp (1961) for *Chiropsalmus buitendijki*. Fortunately, in the local Colombo Museum, I was able to find the original description by Menon (1930) with illustrations, and confirm the specimens as being the same as Menon's *Chiropsalmus buitendijki*. However, work by Southcott (1967) suggested that, because of the arrangement of the tentacles arising from the pedalia, it should be placed in new genus. Consequently he renamed this jellyfish *Chiropsoides buitendijki*, a nomenclature that will be followed here. In most chirodropids the tentacles develop and multiply from the pedalium by bifurcation, and are often in pairs. In *Chiropsoides* the pedalium extends inwards towards the bell and the tentacles develop from this extension in a row in linear fashion.

Distribution

Described first off Java (Horst 1907) and later from Malaysia, Burma, Indo-China, and south India (Menon 1936; Rajagopal *et al* 1989) and now identified by the author in Sri Lanka.

Appearance

A box-shaped bell up to 110 mm bell diameter, with groups of up to 8--9 tentacles in each corner, coming off in an unusual linear fashion on the outside of the main

canal. There is also a very characteristic extension of the pedalial canal extending to the top of the pedalium (which occurs in no other jellyfish described to date). Eight long, superior, perradial gonads hang down to the level of the sensory organs – almost the full height of the bell. They have a similar diameter through the majority of their length in the freshly preserved specimen (although the gonads on some specimens come to a point after preservation in formalin saline).

Sting

Despite chirodropid specimens having been caught by Dr Fernando whilst diving in deep water within a few hundred meters of a swimming beach, lifesavers who patrol these beaches in Sri Lanka have never had to treat any severe jellyfish stings. Until we have further information, it must be presumed that they are a deep-water species and, unlike *Chironex*, do not come into shallow water, or do not cause stings to unwary swimmers.

Chironex fleckeri

Although many specimens of *Chironex* have been studied by the author, the only change to current printed information was that a specimen 25cm in bell diameter had been found on a Mackay beach in April 1997 (see Figure 7).

This size was increased when a specimen 30cm across the bell had been discovered in Port Douglas, north Queensland a few days later. (R Hoare, 1997, personal communication). This is somewhat larger than the previously stated maximum bell diameter of 22cm “or more” stated by Hartwick (1991a).

Table 1 - Comparison of studied characteristics in chirodropids

see also, Figure 6 – chirodropid anatomy

Chirodropid morphology comparisons

Feature	***Chironex fleckeri***	***Chiropsoides buitendijki***	***Chiropsalmus* sp.**	***Chirodropus gorilla***
Bell size	up to 300mm + in width	up to 100mm in width	up to 100 mm in width	up to 130mm in width
Proximal pedalial canal angulation	rose-thorn shape	long, sweeping upward extension	rounded to volcano-shaped	rose-thorn shape
Tentacles	flattened	round	round	round
	relatively robust	fairly robust	robust-fine	robust
	up to 15 per pedalium	up to 9 per pedalium	up to 9 per pedalium	up to 11 per pedalium
Lateral gonad	webs	sheets	sheets	sheets
Superior gonad:				
1. Perradial cores	kidney-shaped	8 long `globules’	rounded	rounded
2. Perradial eminences	cock's-comb bunches of fingers	mammiform	mammiform	grape-like clusters at free edge
3.reproductive tissue	covers perradial eminences	covers perradial eminences	covers perradial eminences	covers perradial eminences
Gastric diverticula	envelops perradial eminences	envelops perradial eminences	envelops perradial eminences	envelops perradial eminences

(author’s findings, not necessarily agreeing with previously-published characteristics)

5.1.3 Physalia morphology

(see Appendix C)

In October 1991 vast numbers of a large *Physalia* sp. previously unrecognised in Australia, were beached in north Queensland (Fenner *et al* 1993b). Stings from these *Physalia* caused severe skin pain and were usually followed by systemic symptoms including nausea, vomiting, abdominal colic, limb muscle cramps, anxiety, restlessness and chest pain (the latter due to intercostal and back muscles myalgia resulting in inspiratory pain cutting off the breath with a "grunting" noise).

When examined by the author, they were morphologically different to the smaller, familiar Australian *Physalia* or "bluebottle" (Fenner *et al* 1993b), which usually have a float length about 3-8cm, but up to 10cm, and with only a single, long "main" tentacle (Williamson 1985a, p4). Occasionally they can also have several long, thin, tentacles, which may not be noticed. The "newly-recognised", larger *Physalia* sp. had float lengths of up to 15cm with up to 5 thick "main" tentacles, usually a dark blue in colour, and up to ten other long, thin, pale-coloured tentacles.

Because of these morphological differences to the common, Australian *Physalia* ("bluebottle"), the author suggested that they should be referred to as *Physalia physalis*, as they were similar morphologically to (although smaller than) the Atlantic species commonly referred to as the "Portuguese man-o'-war" (Fenner *et al* 1993b). The Atlantic *P. physalis* is larger than the Australian specimens with a float length of up to 25cm, and has multiple long, thick main tentacles which have been reported to reach a length of 30m (Halstead 1988). Contrary to Totton's suggestion previously discussed (Totton & Mackie 1960), particularly as the species were very different in their envenomation effects, I suggested that the smaller, single-tentacled jellyfish, commonly known as the "bluebottle" should be referred to by the original name *P. utriculus,* and the newly-described species as *P. physalis*, with the common name "Pacific man-o'-war" (Fenner *et al* 1993b).

Further to this, medical differentiation is necessary, or advisable. *Physalia utriculus* ("bluebottle") stings cause immediate skin pain and wealing, which is quickly and efficiently relieved with cold packs or ice. Some pain felt in the regional lymph glands and occasionally systemic symptoms including nausea, lethargy and dyspnoea have been reported (Williamson 1985a, p4). In contrast, victims stung by the Australian *Physalia physalis* experienced severe skin pain, with only slow relief from cold packs. The stings often caused a mild Irukandji-like syndrome (Fenner *et al* 1993b).

This pattern of symptoms, lasting many hours has previously been described in the multi-tentacled *Physalia physalis* on both sides of the Atlantic (Halstead 1988; Burnett & Fenner, in Williamson *et al* 1996, p198). It has also caused 3 human fatalities in the south-eastern sub-tropical areas of the United States (Stein *et al* 1989; Burnett & Gable 1989), where up to 100,000 stings may occur each year (Fenner *et al* 1993b). Similar symptoms, lasting about 1 hour, have also recently been described in victims stung by the multi-tentacled *Physalia* in Karachi, Pakistan (Database 5.7.5).