CHAPTER 1. GENERAL INTRODUCTION

1.1 Thesis Outline

The thesis is divided up into five main chapters. Chapter 1 consists of a literature review of all aspects of the Anaspidacea including the aims of the thesis, a review of the biodiversity, history, distribution, fossil record, morphology, physiology and ecology. This chapter finishes with a list of the described species prior to this study, and a review of the position of the Super Order Syncarida within the Subphylum Crustacea and the systematic position of the Anaspidacea within the Syncarida.

Chapter 2 is the methods section for the techniques used in the field and laboratory ad data acquisition. The process used in the compilation of the Anaspidacean Site Records used in the distribution mapping is described. The abbreviations used are listed for the institutions from which samples and data were collected as well the abbreviations used for the anatomical features. Definitions are provided for the morphological terminology used as well as the habitat terminology used in this study.

Chapter 3 is the first component of the results section where all species, genera and family classifications are re-examined, re-described with illustrations, and standardised for four of the six families. This section (and Appendix 1.) is used as the basis on which the 'Character Matrix' for the phylogenetic analysis presented in Chapter 4 is developed. The families presented in this chapter are those that present new taxa and therefore new taxonomic data. The last two families, the Patagonaspididae and the Stygocarididae were also reviewed in the same way, however as they did not present new taxa they were separated and presented in Appendix 1. Although no new taxa were added, the Stygocarididae section does present new records from Tasmania, where they had not previously been recorded. Chapter 4 is the second component of the results section. This section involves the development of the 'Character Matrix' used in the phylogenetic analysis of the morphological features of the Syncarida including the extant Anaspidacea, the extinct Palaeocaridacea as the ingroups, and the extant Bathynellacea as the outgroup in the analysis. The methodology and results of the analysis are presented.

Chapter 5 is the last chapter of the thesis and presents a discussion of the phylogenetic tree and the inferred evolutionary pathways and processes developed from the analysis in chapter 4. This is followed by a revised classification of the Anaspidacea and a discussion of the biogeographic implications of the Systematics and Phylogenetics of the Anaspidacea. The final section of the thesis is the conclusions drawn from the study and a discussion and recommendations of further work.

The final sections of thesis include the references and the Appendices. The appendices include the revisions of the Patagonaspididae and Stygocarididae, the Morphological Analysis Character Coding Table, and the list of the Anaspidacea and Palaeocaridacea Site Records.

1.2 A Review of the Order Anaspidacea (Crustacea: Syncarida)

Introduction

The Anaspidacea Calman 1904 is a small order of diverse, southern hemisphere eumalacastracan crustaceans that belongs to the Superorder Syncarida Packard 1885. The Syncarida also includes two other orders, the extinct Palaeocaridacea Brooks 1962, and the extant cosmopolitan Bathynellacea Chappuis 1915. The Syncarida is one of the most interesting invertebrate groups found in Australian inland waters (Schram 1984, Williams 1980). This is because they are an ancient group that branched off from the main lineage of the Eumalacostraca or higher Crustacea at a very early period perhaps as far back as the Late Devonian (400-380mya) with extant taxa still retaining a primitive body structure today. This body plan is characterised by a complete lack of a carapace and unspecialised, highly segmented thoracic and abdominal appendages.

The Anaspidacea is an entirely freshwater order, which currently includes six extant families, 12 genera and 21 described extant species and two monospecific fossil genera. They have a distinctly Gondwanan distribution with five of the families including the Anaspididae Thomson 1893, Koonungidae Sayce 1908, Psammaspididae Schminke 1974a and the new family, Raptornungidae (Family A Serov 2002), being endemic to SE Australia. The Stygocarididae Noodt 1963b currently occurs in SE Australia, southern South America and the South Island of New Zealand while the family Patagonaspididae Grosso & Peralto 2002 is endemic to southern South America. The two fossil species assigned to this order occurred in SE Australia although there is also a possible connection with the fossil *Clarkecaris brasilicus*, Clark 1920 from Brazil that has been suggested to belong to the Stygocarididae (Schram 1984). The distributions of all taxonomic levels are generally mutually exclusive in distribution. This paper presents a review and inventory of all 21 previously described species of freshwater Anaspidacea and the 25 species of fossil Palaeocaridacea.

The Anaspidacea are characterised by having the following features: no carapace; antennae with a single flagellum; antennula with two flagella; compound eyes, when present; seven thoracic segments (which separates them from the other two orders); six abdominal segments; an elongate semi-cylindrical body with thin, flexible integument and range in body size from 0.55mm to 63mm. Each body segment has appendages (that are progressively reduced or absent in the abdominal segments in subterranean species), with the last segment having a telson and two uropods. Colouration occurs only in species with epigean connections whereas subterranean species are generally unpigmented. The sexes are distinctive. The females have no brood pouch and deposit eggs singly onto the substrate. There are no drought resistant life stages or diapause strategies in the eggs therefore they are restricted to habitats with permanent water.

Although originally considered as a solely surface water group occupying lakes, streams and wetlands, the Order is actually predominantly subterranean occupying environments including caves, deep and shallow hypogean groundwaters and hyporheic sediments in river beds across their entire range. They are regarded as one of the dominant flagship groups within the entirely groundwater dependent stygofauna community within south eastern Australia (Eberhard &Spate, 1995; Thurgate, *et al.* 2001a, Thurgate, *et al.* 2001b; Serov 2002).

South East Australia is the biodiversity epicentre for the extant species of Anaspidacea with five out of the six families being endemic and occupying the greatest range of aquatic habitats. The type and range of habitats change along a climatic continuum, generally based on temperature, from the permanently cold, pelagic surface waters of ancient highland lakes and river benthos in Tasmania, to the intermediate surface/subterranean environments within the permanent, seepages zone wetlands and crayfish burrows of , Southern, and Northern Tasmania and throughout southern Victoria to entirely subterranean groundwater habitats such as caves, the hyporheic zone of rivers and the phreatic environment within aquifers as far north as northern New South Wales. The specific surface and subsurface aquatic environments in which anaspidaceans are found are characterised by the persistence, longevity, and stable environmental variables of the water source. These habitats have been described as relictual environments, particularly in an Australian climate context (Williams 1980, Schminke 1982).

Extant taxa whose lineage can be traced through many geological periods via a fossil record are often called `living fossils' (Jarman & Elliot 2000). These rare groups and their associated fossil lineages have stimulated interest from scientists for over 200 years due to information that can be interpreted from their distribution, ecology and the relationships inherent within their morphological and genetic evolution. The consistency and changes in traits combined with geological time, the environmental variables past and present, inform us on the rates of speciation and the suggested evolutionary pathways of not only this group of organisms but also of the associated community and continents.

The concept that the anaspidaceans represent a direct link with ancestral crustaceans has become an entrenched view with many of the morphological characters seen in the Anaspididae being classified as plesiomorphic or primitive. This includes characters such as the lack of a carapace to protect the gill structures, the leaf-shaped thoracopod gills, and a basic eumalacastracan body plan (Brooks, 1969) with relatively unmodified trunk and appendages (Dahl 1983). As a result, the Anaspidacea and Syncarida have generally been regarded as a basal or `primitive' lineage within the Malacostraca (Williams 1980; Dahl 1983; Wallis & MacMillan 1998).

Over the last 120 years, since the first description of *Anaspides tasmaniae* by Thomson in 1893, there have been two major problems with the taxonomy of the Anaspidacea that have created inherent barriers in understanding the interrelationships within the group. The first is the overall similarity and 'apparent' lack of easily distinguishable morphological characters with which to separate the taxa within each of the families. This is the main factor that has contributed to a significant underestimation of the biodiversity of the Anaspidacea. This has led to previous workers relying almost completely on a few gross external features or 'gestalt' to separate the species and genera rather than critically analysing fine detail variation in character states. The characters on which the taxonomy was based to separate the two described species of *Anaspides* have been questioned by several authors (Horwitz 1989; O'Brien 1990; Eberhard *et al.* 1991) although they did not examine the species morphology in detail and based their opinions solely on telson structure.

These generalised assumptions of what constitutes a species have been further reinforced by the Anaspidacea possessing highly conservative genes (Jarman and Elliot 2000) and inhabiting environments that have been stable both in structure and conditions for millennia (relictual habitats), thus resulting in little need for major morphological change. The conservative nature of their morphology has resulted in many highly cryptic species (Jarman & Elliot 2000) being classified as broad multi-catchment, single species or even single transcontinental genera. The three genera that have encountered the most difficulty in delineating species are *Anaspides* (Anaspididae), *Koonunga* (Koonungidae) and *Stygocaris* (Stygocarididae). The broad distributions in the lower taxonomic levels appear entirely incongruent for a group that is: entirely restricted to freshwater surface, and particularly groundwater, habitats; have limited or no overland means of dispersal; has narrow physiological tolerances; and no life history strategies to tolerate drought.

This apparent homogeneity of form and an apparent low number of species has been caused to a large extent by the second problem, that of generally short, insufficiently detailed illustrations and descriptions and the use of ambiguous, non-diagnostic characters by most authors. These problems have resulted in the masking of the high species diversity of this group. *Anaspides tasmaniae* is a classic example of the previous misconceptions of species range and species defining characters. *Anaspides tasmaniae* was first described in 1893 from the alpine streams on Mount Wellington, near Hobart, Tasmania. Since that time all *Anaspides* specimens from across Tasmania have been placed within this species, with the exception of one lake dwelling species (*Anaspides spinulae*, Williams1965). In the most recent re-examination of the family Anaspididae and *Anaspides tasmaniae*, Jarman & Elliot (2000) stated that the species exhibit morphological stasis or stability. In this study they identified 3 species clades using 16SRNA analysis and exemplified the morphological uniformity of this group. They did not however attempt to examine the specimens taxonomically as they considered them too similar in overall appearance. That fact that they identified these groupings as both species *and* clades is inherently contradictory in itself as they could not decide or were not confident that the

techniques were adequate to separate the specimens examined into species or genera. If this level of diversity is exhibited within a surface group, the subterranean group will certainly be even more diverse and locally endemic. This work demonstrates that 1) the techniques used were not as robust in separating species as they first thought and 2) that there was a higher biodiversity of species than could be separated by the taxonomic characters used at the time. This throws into doubt the species assessments of the past and points towards a family composed of genera and species with far more limited distributions than previously thought. Although this is not an isolated problem in the identification a small crustaceans, it appears to be a common misconception with regards to *A. tasmaniae* and the Anaspidacea in general.

Despite extensive collections of Anaspidacea (refer to Appendix 2.) being housed in various museum collections from over 100 years of collecting only a small number of species have sufficiently detailed descriptions to separate genera from species and only four have any detailed ecological or life history information available. In terms of life histories, Hickman (1937) provided the first insights into the age and life cycles of the type species *Anaspides tasmaniae* from the type locality, Mt Wellington. Williams (1974) provided a preliminary analysis of *Anaspides tasmaniae* from the data available at the time including his report from 1965. Swain & Reid (1983) conducted a more detailed analysis of the population structure and life history of Anaspides from the Mt Field area with a comparison study conducted at Silver Falls on the southern slopes of Mt Wellington by Serov (1988). Fulton (1983) presented a brief description of the possible life history of *Paranaspides lacustris*. More recently two detailed studies on the population and life histories were presented for both *Allanaspides helonomus* (Swain 2000) and *Allanaspides hickmani* (Driessen *et al*. 2007). A more detailed summary of these studies will be presented later in this chapter.

This study presents a morphological investigation instead of a genetic analysis due firstly to the lack of specimens suitable for genetic analysis as a result of age and inappropriate preservation. Most collections in museums are in excess of 50 years old (often much older) and were often unsuitably preserved in substances such as formalin. It is evident from the genetic studies such as Jarman and Elliot (2000) and Andrews (1999), especially single gene ones, don't seem to be as reliable as first thought, and are not able to adequately separate genera from species. Most importantly though is the fact that morphological variation evolves over time as a functional necessity in response to environmental change, whether that is climatic, or geological or a combination of both. Therefore, in order to understand more comprehensively the distribution, history and ecology of a species it is necessary to understand the differences in form and the function of that form that define the species, genera and higher taxonomy. It is important not only to know that there has been a change genetically but what that change means morphologically, physiologically and ecologically. It also remains true that new species still require formal morphological description. Defining the physical characteristics in a standardised format will also aid in future environmental management and conservation purposes by providing consistent taxonomic identifications and keys.

Aims

The principal aims of this thesis therefore are to clarify

1) The morphological taxonomy and

2) Discuss the general concepts of the Systematics and Phylogenetics of the Anaspidacea based on the morphological variation pathways.

The specific aims of this study include:

- Revising and standardising the morphological characters and taxonomy of all taxonomic levels of the Order Anaspidacea by redescribing known species using type material, where available, and describing new species from collections held by museums, government agencies, the literature, other researchers and new collections by the author.

- Investigate the general evolutionary pathways and possible divergence ages for the Anaspidacea based on morphological and distributional evidence from all families.

- Define the species concept in terms of morphological characters and identify diagnostic characters and the potential species ranges for each taxonomic level within this group.

- Demonstrate the evolutionary changes to the morphology, physiology and ecology and the development of the association with groundwater across the Order.

- Provide additional information on the ecology, physiological tolerances and environmental requirements of the group.

This thesis does not aim to identify every new species, of which there is so many that time and space would not permit it. Rather, the aim is to demonstrate the general morphological variation across the Anaspidacea by presenting single species as example of each genus within a family and in some cases, present a small number of species to illustrate the variation within the genera.

Distribution

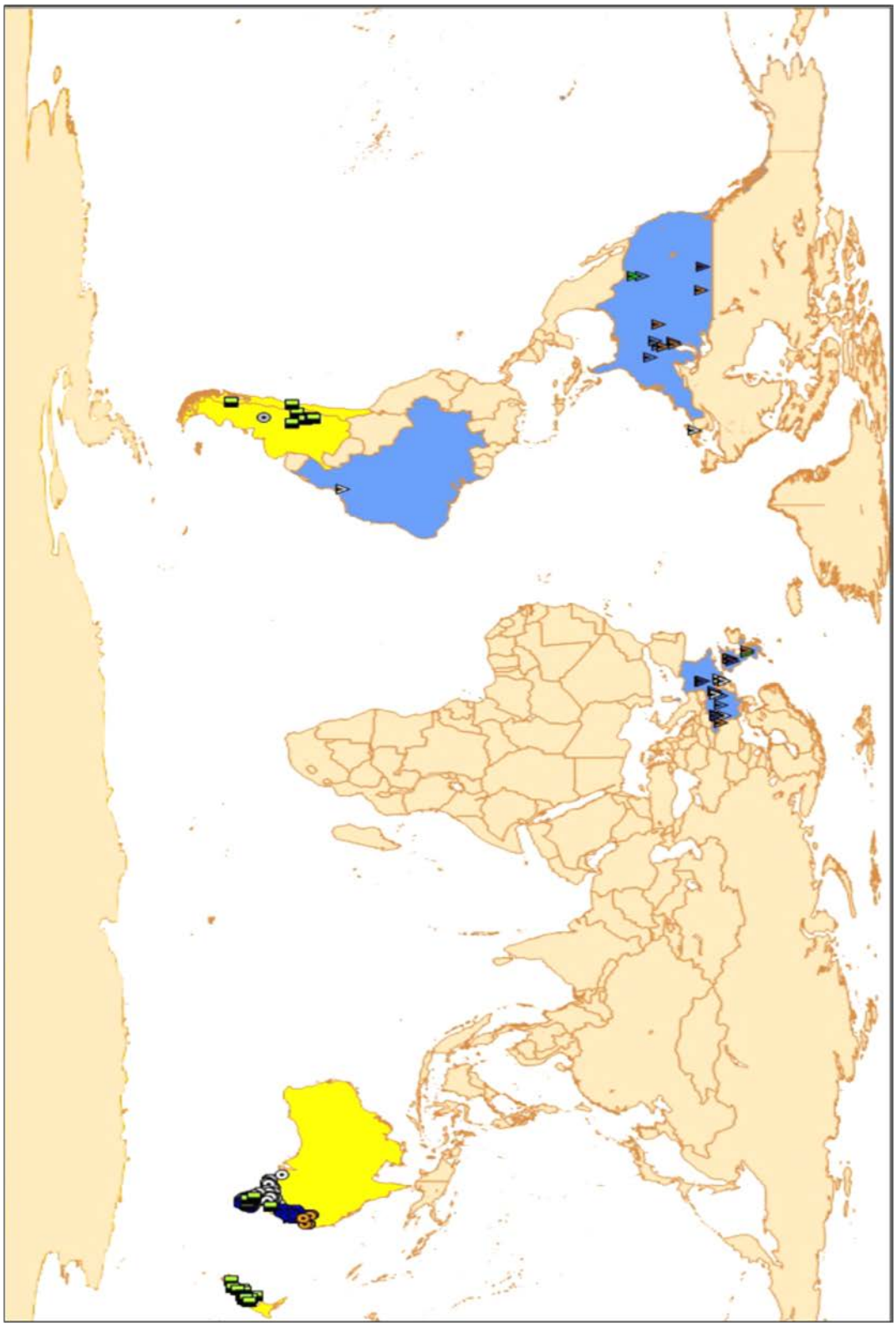
(Maps 1.1 and 1.2)

The Syncarida have been recorded in almost every continent around the world with the exception of Antarctica. This cosmopolitan distribution is attributed mainly to the tiny and ancient Order Bathynellacea which contains three families, the Bathynellidae, Parabathynellidae and the reintroduced Leptobathynellidae (Coineau & Camacho 2013). The Anaspidacea however, has a far more restricted Gondwanan distribution covering southern South America, south-east Australia and New Zealand. In South America they have been recorded from the countries of Chile, Argentina and the southern area of South America shared by both countries known as Patagonia. The South American Anaspidacea belongs to two families, the Stygocarididae and Patagonaspididae, which are currently grouped within the Suborder Stygocaridinea.

Australia represents the epicentre of extant anaspidacean biodiversity with five of the six families described in this study occurring in the southeast corner of the country including New South Wales, Victoria and south-east South Australia and Tasmania. Tasmania has the highest diversity of Anaspidacea families including: the endemic family Anaspididae which includes the iconic *Anaspides tasmaniae* or Tasmanian Mountain Shrimp. This family is distributed across central and southern Tasmania; the Koonungidae occurs on the North West coast; the family Psammaspididae occurs in caves in the central north down to the southwest and includes the only record of Anaspidacea from the northeast of the state; and finally the family Stygocarididae with new records from the north coast to the central Midlands. Victoria contains three families, the dominant Koonungidae which occurs across the state, and one record each of the Stygocarididae recorded in SE Victoria and the Psammaspididae being recorded from the southwest. South Australia has only one family, the Koonungidae, found in caves and permanent spring fed wetlands, whereas New South Wales has two families including the Psammaspididae and the new family Raptornungidae although the most northerly record of the Koonungidae is recorded from the hyporheic zone of the Murrumbidgee River at Albury on the border between NSW and Victoria is technically with NSW.

New Zealand is occupied by just one family, the Stygocarididae. Published records of this family have so far been recorded by Schminke (1978) in which he recorded 36 sites out of 200 (i.e., 18%) freshwater interstitial samples taken throughout the South Island of the country. They were collected from alluvial groundwaters in Nelson, Marlborough, and Canterbury on the South Island and are reported from Hawkes Bay in the North Island (Scarsbrook *et al*. 2003 and G. Fenwick pers. comm.) although precise locality data has not been found. Scarsbrook (2003) also suggested that they are certain to be even more widespread.

The extinct Order Palaeocaridacea appears to have originated in and around Scotland in the late Devonian and has its biodiversity epicentre in Western and central Europe (France, Germany and the Czech Republic) and North America during the Carboniferous (Woodward 1908, Calman 1911, Scott 1938, Brooks 1962, Schram 1979a, b, Schram 1984). The fauna continued to disperse south to central eastern South America and across to NSW in south east Australia by the Triassic with the final record occurring in south east Victoria during the Cretaceous.



Map 1.1. Map of the worldwide distribution of the Anaspidacea and Palaeocaridacea.
Symbol legend: Fossil Palaeocaridacea - ▲closed triangles; Extant Stygocaridinea - ❑ half closed squares; Extant Anaspidinea - ⊙ open circles. Country colours: blue - countries with Palaeocaridacea; yellow - countries with Anaspidacea; grey countries without records. Refer to Appendix 2 for listing of site records. N.B. Alaska does not have Syncarida fossils.

Fossil Record

The Palaeocaridacea are a morphologically diverse and widespread group of fossil Syncarida that formed a significant component of the freshwater and brackish wetland and delta macroinvertebrate communities for over (at least) 200 million years. The first fossils appeared in the Upper Carboniferous (320my) and persisted until the Lower Permian across the northern hemisphere and continued to exit into the Cretaceous (118my) in the lakes and wetlands of South Eastern Australia (Brooks 1962, Schram 1984). The distribution of the Palaeocaridacea is presented in Map 1.2 and a list of the Palaeocaridacea is provided in Table 1.1 below

The origin of the Syncarida and the divergence of the Palaeocaridacea and the cosmopolitan Bathynellacea (and possibly the Anaspidacea) are likely to have occurred in the mid to late Devonian as the orders were already well defined, established and diverse by the Carboniferous (Schram 1984). It is also likely that the separation from surface dwelling species represented by the Palaeocaridacea to the colonisation of the subterranean environment represented by the Bathynellacea (and possibly the early anaspidaceans) also occurred at an early age, possibly as early as the Devonian. The occupation of groundwater environments is one of the possible reasons that the families of Bathynellacea and the Anaspidacea such as the Psammaspididae and Stygocarididae have no fossil record, as well as the fact that their thin, flexible integument would not preserve well as well as the fact that being of small to very small size would make them difficult to detect in geological mediums. The order Palaeocaridacea currently consists of five families, 18 genera and 39 species including six genera without family designations (Family Uncertain). They are distributed across 11 countries including the United States of America, Canada, Scotland and England, France, Germany, Belgium, Czech Republic, Brazil and Australia.

Although many species have been collected in many countries and over a vast span of geological time, the evolutionary pathways for the order are still disjunct with large gaps in the fossil record. This is due predominantly to the lack of the very specific, fine grained anoxic environments that were required to adequately preserve these small animals with very thin and flexible integuments. Most species were benthic/pelagic animals that inhabited the still, freshwater, lacustrine coal swamps/ delta environments that occurred around the world throughout the Carboniferous to Permian (Schram 1984). The only variations to this are: *Clarkecaris brasilicus* Clarke (1920) from Brazil which occurred in shallow marine/estuarine lakes during the Upper Permian; *Anaspidites antiquus* Chilton (1929) was collected shale beds from the sand bed delta of the Sydney Basin during the Triassic and *Koonaspides indistinctus* Jell & Duncan (1986) from a Cretaceous shallow freshwater lake in Victoria, Australia.

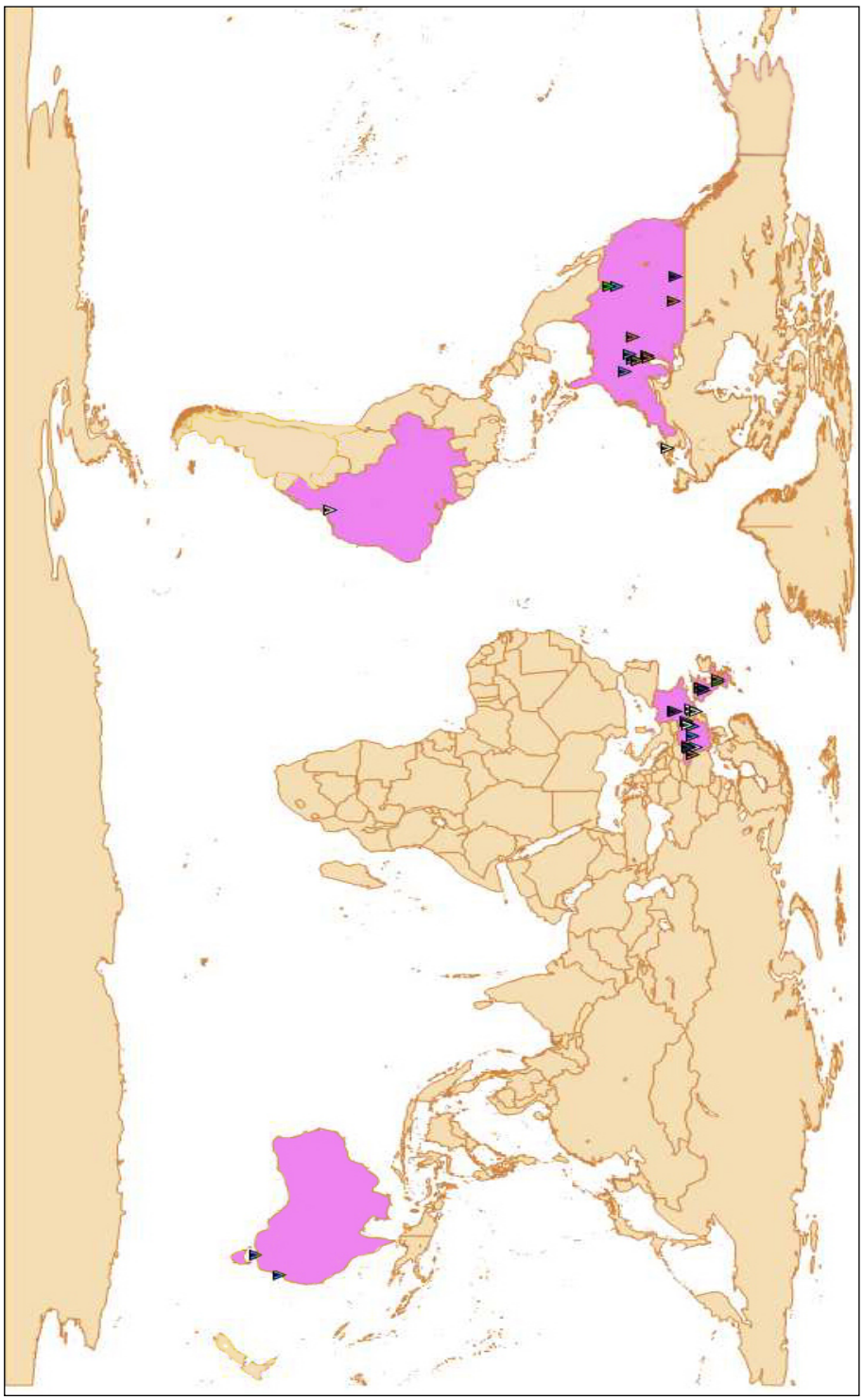
It will never be known how many different environments the Palaeocaridacea occupied however, the variability in morphology suggests it is likely that they occupied a similar range of environments to the

extant Anaspidacea including benthic and pelagic zones within surface water lacustrine lakes and wetlands, the benthos of rivers, and possibly even the subsurface hypogean and hyporheic environments of caves, rivers. They are generally regarded to have similar feeding habits and ecologies to the Anaspidacea (Perrier *et al*. 2003, 2006, Schram 1984)) i.e. being opportunistic detritivores and algal grazers based on similar body and mouthpart (where available, e.g. *Palaeocaris secretanae* (Perrier *et al*. 2006)) structures and ornamentation although several species have well developed anterior grasping maxillipeds and thoracopods suggesting they may have been active predators or opportunistic carnivores or scavengers such as *Acanthotelson stimpsoni*, *Palaeosyncaris dakotensis* and *Palaeosyncaris micra*.

The Palaeocaridacea and the Anaspidacea are remarkably similar in body structure however, they differ morphologically in a number of key features. These include the number of thoracic segments and the shape of the uropods and shape of the telson. Palaeocaridacea are defined by the eight thoracic segments, which they share as a plesiomorphic character with the Bathynellacea, typically both uropodal rami are a single segment, elongate paddle shaped appendage and typically the telson is elongate and triangular and as long as the uropods. The Anaspidacea differ by having only seven thoracic segments, both uropodal rami ranging from two to one segmented, narrow, elongate to leaf shaped rami, and small to medium rounded to triangular rostrum, which are much shorter than the uropods.

These fossil species are divided into two main groups in terms of distribution and geological age. The first and major group of Palaeocaridacea (*sensu stricta*) occupied the rivers, deltas and coal swamps of Europe (England, Scotland, Germany, France, Czech Republic and the Netherlands) and North America (USA and Canada) for approximately 30 million years between 330 to 299mya. This was followed by a break of 22 million years in which no fossils have been found and following on from this period no further Palaeocaridacea are recorded from Europe or the USA therefore signalling the extinction of this fauna from the Northern Hemisphere. The period just before and after this break also signalled a change in body style from the body segments being either all subequal (the plesiomorphic form) or the abdominal segments being shorter than the thoracic segments to one where the thoracic segments are shorter than the abdominal segments. The second group of Palaeocaridacea is recorded from Brazil in South America (*Clarkecaris brasilicus*) and two species from South East Australia. This second group existed from 278 mya to 118 mya covering 160 mya. Although only a small number of specimens and species exist for this period it demonstrates: firstly, a migration from the northern to the southern hemisphere; and secondly that they existed across South America and South East Australia. The fact that extant species of Anaspidacea (Stygocaridacea) still occur in South America, Australia and New Zealand today would indicate that 1) the Anaspidacea had split from the Palaeocaridacea prior to the breakup of South America and New Zealand from Antarctica (130 mya) and 2) would have followed the same distributional pathways to Australia via Antarctica as the remaining species of Palaeocaridacea.

The Palaeocaridacea that have significant affinities with the Anaspidacea occur in several different Palaeocaridacean families known from both the northern and the southern hemispheres (Brooks 1962; Schram 1984, Jell & Duncan 1986). Two fossil species have also been included within the Anaspidacea. *Anaspidites antiquus* Chilton 1929 was the first to be classified within the Anaspididae. The species was identified from fossils in the mid-Triassic (240 Ma) Wianamatta Shales of the Hawkesbury Sandstones of New South Wales, Australia, and was considered to be morphologically almost indistinguishable from *A. tasmaniae* (Chilton 1929; Brooks 1962; Schram 1984). This discovery contributed to the' living fossil' status of *A. tasmaniae* as it demonstrated that at least the Anaspididae have sustained long periods of morphological stasis. The second species to be included in this family is *Koonaspides indistinctus* described by Jell & Duncan in1986 from the Koonwarra Insect Beds from Cretaceous (Koonwarra118-115 mya) deposits in Gippsland, Victoria, Australia.



Map 1.2. Distribution of the fossil Syncarida.

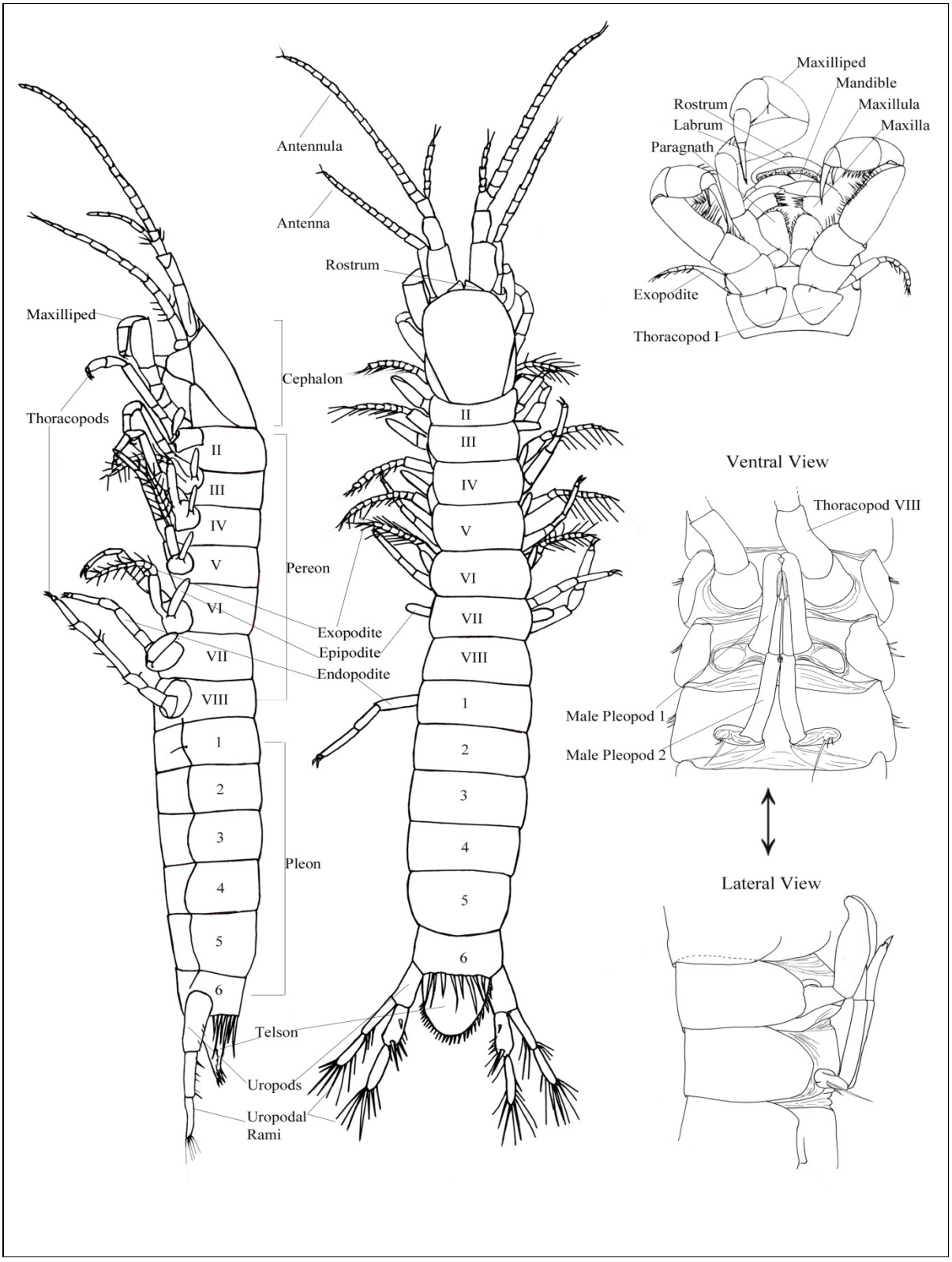
Country colours: purple - countries with Palaeocaridacea; yellow - countries with Anaspidacea; pink countries without records. The exception to these rules is Australia where the blue is not defined for the fossil areas and Alaska that has no record of Palaeocaridacea.

Family	***Species***	**Habitat**	**Country**	**Author & Date**	**Age (MYO)**
Acanthotelsonidae	*Acanthotelson kentuckiensis*	Fresh to Brackish water	USA	Schram 1984	311
Acanthotelsonidae	*Acanthotelson stimpsoni*	Fresh to Brackish water	USA	Meek and Worthen 1865	310
Acanthotelsonidae	*Uronectes fimbriatus*	Fresh to Brackish water	West Germany	Jordon 1847	299
Acanthotelsonidae	*Uronectes kinniensis*	Fresh to Brackish water	USA	Schram and Schram 1979	305
Acanthotelsonidae	*Uronectes palatinus*	Fresh to Brackish water	Germany	Uhl 1999	313
Minicarididae	*Eurythrogaulus carrizoensis*	Fresh to Brackish water	USA	Schram 1984	299
Minicarididae	*Minicaris brandi*	Fresh to Brackish water	Scotland	Schram 1979	330
Palaeocarididae	*Monicaris rubnicensis*	Freshwater	Czech Republic	Stamberg 2000	299
Palaeocarididae	*Palaeocaris retractata*	Freshwater	England	Calman 1932	313
Palaeocarididae	*Palaeocaris secretanae*	Freshwater	France	Schram 1984	306
Palaeocarididae	*Palaeocaris typus*	Freshwater	USA	Meek and Worthen 1865	315
Palaeocarididae	*Palaeosyncaris dakotensis*	Freshwater	USA	Brooks 1962	320
Palaeocarididae	*Palaeosyncaris micra*	Freshwater	USA	Schram 1984	310
Squillitidae	*Nectotelson krejcii*	Freshwater	Czech Republic/France	Fritsch 1875	299
Squillitidae	*Praenaspides praecursor*	Freshwater	England	Woodward 1908	313
Squillitidae	*Squillites spinosus*	Fresh to Brackish water	USA	Scott 1938	320
Anaspididae	*Anaspidites antiquus*	Freshwater	Australia	Chilton 1929	240
Anaspididae	*Koonaspides indistinctus*	Freshwater	Australia	Jell & Duncan 1986	118

Family Uncertain	*Brooksyncaris canadensis*	Freshwater	Canada	Brooks 1962	315
Family Uncertain	*Clarkecaris brasilicus*	Marine to brackish water	South America	Clarke 1920	278
Family Uncertain	*Palaeorchestia parallela*	Freshwater	Czechoslovakia	Fritsch 1876	299
Family Uncertain	*Pleurocaris annulatus*	Freshwater	Belgium	Calman 1911	313
Family Uncertain	*Pleurocaris juengeri*	Freshwater	Germany	Schöllman 1999	313
Family Uncertain	*Spinocaris horribilis*	Freshwater	Germany	Uhl 1999	313
Family Uncertain	*Williamocalmania vandergrachti*	Freshwater	The Netherlands	Pruvost 1912	320

Table 1.1. Species list of the Palaeocaridacea.

Morphology and Physiology



Chapter 1 Figure 1.1. Generalised external anatomy of the Anaspidacea. Psammaspididae: Left side - whole animal with lateral and dorsal views; top right - ventral view of cephalon illustrating the mouthparts; Right middle - ventral view of male petasma; Right bottom - lateral view of male petasma.

External Anatomy

The Anaspidacea are distinguished from other crustacean groups by having the following characteristics. The head is subrectangular, longer than wide and does not possess a carapace. The head in anaspidaceans is formed by the fusion of the cephalon with the first thoracic segment. The structure of the cephalon in the Anaspidacea consists of a frontal lobe and a number of lateral and dorsal grooves. The retention, reduction or loss of these grooves on the cephalon is diagnostic at the family level. There are seven types of cephalic grooves that have been recorded for the Syncarida with five occurring in the Anaspidacea to varying degrees and at various stages of development.

The types of grooves that occur with the Anaspidacea include: the medial groove; rostral groove; mandibular groove (Mandibelfurche, Siewing 1959); cervical groove (Cervikalfurche, Siewing 1959); and the horizontal groove or Horizontalfurche (Siewing 1959). The Palaeocaridacea have a least two additional grooves that include the precervical groove found in *Acanthotelson stimpsoni* (Schram 1984) and the postcervical groove described in *Brooksyncaris canadensis* (Schram 1984). The development of the grooves was first described by Hickman in 1937 with his examination of the embryonic development of *Anaspides tasmaniae.*

Cephalic grooves first appear in the embryonic larval and newly hatched stages of *Anaspides*. The medial groove is the first to form in the embryo stage and separates the developing eyes. Prior to hatching the rostral groove forms to join the eyes and the medial groove, which subsequently separate to form the stalked eyes of the adults. The next groove to develop is the cervical groove that forms a straight line laterally across the whole cephalon separating the frontal lobe from the posterior portion of the cephalon. As the larva develops the central margin of the groove migrates posteriorly forming a rounded v-shaped groove with the four celled dorsal organ forming anteriomedially to it (Hickman 1937). In adults Anaspididae, this structure is retained however, both Coineau & Camacho (2013) and Siewing (1959) state that in this case the cervical groove equates to the mandibular groove. The last groove found within the Anaspididae is the horizontal groove. It is a horizontal to diagonal groove on the lateral margin joining the mandibular groove with the posterior margin of the cephalon. It is also found within *Anaspidites*. It is suggested here that may also be a homologous groove to the precervical groove found in *Acanthotelson stimpsoni*. Within the sister Order Bathynellacea as the first thoracomere is unfused there is only the mandibular groove (Coineau & Camacho 2013)

Eyes are variable across the order ranging from highly flexible compound eyes positioned on stalks on each side of the small rostrum (Nilsson 1990), small numbers of sessile ocelli on the dorsolateral margin of the head, (Sayce 1907, Calman 1908, Bowman 1984, Leys *et al*. 2005) or absent completely due to their interstitial lifestyles (Nicholls 1931, Noodt 1963b, Schminke 1974). In the Anaspididae, the optic design is a refracting superposition type (Nilsson 1990). The eye stalk in *Anaspides tasmaniae* contains the Organ of

Belonci, which is described by Kauri and Lake (1972) as either a photoreceptor, perhaps similar to the epiphysis of in vertebrates or a chemoreceptor with active secretary functions. The stalked eye of the Anaspididae provides the animal with greater flexibility and a large panoramic field of vision due to the eye being mounted on a long stalk that protrudes from the optic groove, as did the Palaeocaridacea (Perrier et al. 2006).

The cephalon of all species possess a structure that is universally referred to as a small rostrum. In this study the rostrum is defined as any projection from the anteromedial region of the cephalon although it typically refers to an anteromedial projection of the cephalic plate. In the Anaspidacea the rostrum is formed by either a single triangular lobe or extension of the cephalic plate, or a single, rounded, triangular or bilobed articulated lobe formed from the anterior margin of the vertex beneath the cephalic plate. Rostrums vary in shape from bilobed rostrums with a central groove separating the lobes to broad and narrow, triangular rostrums formed by an anterior extension of the cephalic plate. The cephalon structure of *Allanaspides* is similar to *Anaspides* with the exception that it possesses a transparent oval plate on the top of the head termed the 'fenestra dorsalis' on the anterior dorsal surface of the cephalon (Swain et al 1970, Swain et al 1971, Swain & Lake 1974, McConnell 1987, Lake, Swain & Ong 1974). The Anaspididae and in particular, *Anaspides*, also possess two types of organs on the cephalon, the Nucal organ and the Dorsal organ. The Nucal Organ is a clear spot of thin cuticle surrounded by a dark ring. This organ is specific to the *Anaspides* and *Paranaspides* and is located in the median region forward of the mandibular suture or furrow (Coineau & Camacho 2013). The Dorsal organ is a pore surrounded by up to four dark spots on the medial posterior region of the cephalon (Coineau & Camacho 2013). This is present of all Anaspididae however, is most prominent in *Anaspides*.

Anaspidaceans range in size from a maximum 63mm for the largest *Anaspides* to less than 1mm in the Stygocarididae. The body of the Anaspidacea consists of seven, parallel, free thoracomeres. The first thoracic appendage is modified as an enlarged maxilliped. It is modified as a feeding structure. The thoracic appendages from two to eight consist of a jointed endopodite that function as walking legs. They also possess respiratory epipodites, which originate from the outside margin of the coxa, and vary in shape from the flattened leaf shaped gills found in the Anaspididae to elongate, sausage shaped gills found in the remaining families. The thoracopods also possess highly articulated feather like exopodites, which originate from the basis, and function for water circulation across the epipodites. The exopodites are not always present on the posterior legs.

The leg appendages of the Syncarida including the Anaspidacea are the least modified of any of the Eumalacostraca. Reductions in epipodites and exopodites in a number of families, particularly, all of the extant families and the Bathynellacea, except the Anaspididae have evolved during the colonization of the interstitial environments (Watling 1983).The Mysidacea, Lophogastrida and Euphausiacea have modified

branchial epipodites while the Thermosbaenacea, Spelaeogriphacea, Tanaidacea and Cumacea are possibly the most advanced taxa by possessing highly modified endopodites and no exopodites.

The abdominal section of the body consists of six, parallel pleomeres, each with a pair of pleopods. The only exception is *Paranaspides lacustris* that has a dorsally expanded/wedge shaped first pleomere that gives the body a dorsoventral flexure. This flexure is also present to a lesser degree in *Allanaspides*. The pleonites each have ventral pleopods although their presence and degree of development varies from multisegmented exopodites, to reduced single articles, or entirely absent, principally depending on their adaptation to subterranean environments. The Palaeocaridacea also have a variety of pleopods that range from multisegmented to bi-rami appendages (with an endopodite and exopodite) to narrow, elongate, single article, paddle shaped appendages (Schram 1984). The pleopods in the Anaspididae also possess small, round coxal epipodites. These are absent from all other families. The last pleonite (6) contains a telson and lateral uropods of variable shape and size. In the Anaspididae, the telson is small triangular to rounded and the uropods consist of a single short peduncle and two single segmented rami with a broad, flattened leaf like shape. The telson and the uropods together form a tail fan. In all other families, the uropods are elongate, rounded to horizontally compressed with between one - two rami on either the endopodite or exopod and the telson can be either short or elongate (Schminke 1976).

The structure of the antenna and antennula is relatively consistent across the order and includes an antennula with three segmented peduncle with biramous flagella of unequal lengths including an elongate multisegmented flagellum and a short flagellum. The first segment possesses a statocyst. The statocyst is a small pouch or sac that opens on the dorsal surface through a small orifice and contains club or ball setae termed median crista static. These setae have an articulated stem with large club-like or round setae. According to Coineau & Camacho (2013) there are no statoliths, instead the sac contains mineralised balls on a double articulated shaft set at less than 45º and numerous innervated sensory setae at its base (Coineau & Camacho 2013). *Anaspides tasmaniae* has 23 setae (Coineau & Camacho 2013) whereas the remainder of the families have two-three setae. The movements cause the clubs to push against the setae when the animal moves. The deflection of the setae provides feedback to the animal on changes in orientation, allowing balance to be maintained and, particularly in subterranean organisms, the ability to determine up or down (Cohen 1960).The antenna consists of a four segmented peduncle with multisegmented flagella. An antennal scale or scaphocerite is present on the lateral margin of the second segment of the antenna within the Anaspididae and many of the Palaeocaridacea but is absent from the other anaspidacean families.

The mouthparts include a labrum, mandible, maxillule, maxilla, paragnath and the maxilliped. The labrum is a broad, rounded plate that is positioned ventral to the rostrum and anterior to the other mouthparts. The mandible in the anaspidaceans includes a lateral, serrated incisor process, and a molar process with a setose

or tuberculate lobe. A spine row was initially suggested to be present between the incisor and molar processes with the Anaspidacea (Knott & Lake 1980) however, when compared with the spine row of the Pericarida, the Anaspidacea typically does not possess this feature (Gordon 1961). The only species that contain any structure in this location is the Stygocaridacea which possess 2-3 elongate bristle tipped setae (Gordon 1964). The mandible of the Anaspididae does possess one-two large medially terminal denticles at the end of the incisor process however the author considers this simply the terminal denticle of the incisor process. *Anaspides* also possesses a small row of fine setae extending from the lateral end on the molar process that may be interpreted as a residual spine row however, this is considered as part of the molar process and not a separate spine row. Schminke (1974b) suggested that the proximal part of the incisor process might represent a lacinia. In *Anaspides*, Gordon (1961, 1964) was inclined to homologize a small serrate process between the incisor process and the spine row with the lacinia mobilis of Lophogaster. In a minority of cases (within the Bathynellacea) the distal most spine in the spine row may become articulated and assume the shape of a peracarid lacinia mobilis (Dahl and Hessler 1982), therefore the Anaspidacea (and Syncarida) are considered not to have a lacinia mobilis (Coineau & Camacho 2013).

The mandible also possesses a three segmented palp on the lateral margin in all families except Patagonaspididae Grosso & Peralto 2002 and the new Raptornungidae (Family A Serov 2002) which lacks a palp altogether. In *Anaspides* the mandible is set at an oblique axis (Watling 1983; Gruner 1996) that allows the ingestion of food particles of different sizes. The structure of the mandibles indicates the ability to slice and grind relatively large food items. The paragnath is a pair of lobes covered with fine setae.

The maxillule and maxilla function to direct food particles into the mouth and/or to sort sediments. Both appendages have numerous marginal setae to capture and filter food. The maxillule consists of a two endites in all families except the Anaspididae, which also includes a small exite. The maxillae consists of a large protopod with three endites with the medial margin lines long plumose setae.

The posterior feeding appendage is the maxilliped. It performs a variety of functions including the capture of prey and handling the food item close to the mouth (Gruner 1996). The Anaspidacea is the only order of Syncarida that possesses a maxilliped due the fusion of the cephalon with the first thoracomere (Coineau & Camacho 2013). The maxilliped consists basally of a 2 segmented protopodite with 2 epipodites, a praecoxa and coxa on the first segment. This is followed by 2 endites that include a six segment endopodite, and an exopodite of 2 segments which has a respiratory function (Coineau & Camacho 2013). The six segments include a basis, ischium, merus, carpus, propodus and dactylus. The dactylus terminates in 4 robust spines or claws. The large size of the Maxilliped would indicate either a burrowing or prey capture function as well as to groom the mouth parts. The labrum and labium also assist in food intake.

Internal Anatomy

Nervous System

The nervous systems in *Anaspides tasmaniae* consists of three nerve centres that includes a supra, oesophageal and ganglion centre. These supply three nerves that include the optic, antennular, and a band of segmented ganglia (Siewing 1959). The thoracic region has eight ganglia (one in each segment). Each ganglion has three nerve pairs: an anterior pair for the appendages; a thin pair for the ventral muscles and a posterior pair for the oblique muscles (Smith 1909a, Siewing 1959). The Syncarida differ from other Eumalacostraca by lacking a sub-oesophageal ganglion (Siewing 1959).

Each side of the nerve cord are two giant fibres that originate form the soma of the last five thoracic ganglia and all six abdominal ganglia (Silvey & Wilson 1978). Each fibre is composed of eleven segments. The giant fibres receive input from the head, thorax and abdomen and are responsible for the caridoid escape response of Anaspides (Silvey & Wilson 1978).

Heart

The heart is located dorsally between the first and eighth thoracic segments and consists of an elongated, narrow tube, which extends through the entire thorax and passes into the abdomen without any definite constriction. The anterior and posterior aortae extend from it (Smith 1909a). There are seven pairs of lateral arteries each having podal and visceral branches (Smith 1909a). The circulatory system found in *Anaspides* is also found within the Mysidacea, Lophogastrida, and Euphausiacea. All other systems, except for the Isopoda, show reductions in this pattern (Watling 1983).

The heart of *Anaspides* is thin walled, tubular and consists of a continuous epicardium covering a single layered myocardium (Hickman 1937). The ultrastructure of the heart is considered by Tyonneland *et al* (1984) to be advanced as the cardiac sarcomeres lack primitive features such as a diffuse z-line or an m-line.

The fenestra dorsalis in *Allanaspides* functions in the absorption of scarce ions from the surrounding environment and then transferred directly into the bloodstream (McConnell 1987, Lake, Swain & Ong 1973, Swain & Lake 1974). This structure may contribute significantly to the active sodium transport system. *Allanaspides* is considered by McConnell (1987) to be idiosyncratic among the Syncarida by possessing an ion transport system that does not decrease its effectiveness in a low pH environment. The suggested explanation for this structure is that the pH level in the blood is lower than that of other crustaceans and the blood potassium concentration is relative high, which may be of importance to its survival in an acidic environment. McConnell (1987) proposed that the fenestra dorsalis may have evolved paedomorphically from a larval organ carry out the tasks assumed by the gills of the adult.

Excretory System

Excretion by anaspidaceans is by a maxillary gland. This gland is located at the base of the maxilla (Manton 1931, Nicholls & Spargo 1932). The system commences with a terminal sac that passes into a coiled tube with a glandular epithelium. The tube enters another coiled tube that opens into a straight think wall of the excretory duct. This duct opens through a pore on the outside of the maxilla (Manton 1931). Maxillary glands are present in Syncarida, Isopoda and the Lophogastrida, whereas Mysidacea, Amphipoda and the Decapoda possess the possibly more advanced antennal glands (Manton 1931).

Digestive System

The digestive system in *Anaspides* consists of the mouth, a foregut, one pair of ostia situated in a constriction in the third thoracic segment. The foregut of *Anaspides tasmaniae* was described by Oshel & Steele (1988) as have a mat of short setae at the anterior end of the foregut. Instead of a pyloric filter basket as found in the Amphipoda and Isopoda, it has a setose median projection into its pyloric stomach. The projection is surrounded on each side by a fold of cuticle with a single row of setae and is suggested act as filter setae (Oshel & Steele 1988). There are also setae in the position of the lateral ampullae as found Lophogastrida, Isopoda and Amphipoda.

The alimentary canal has a simple gastric mill with numerous ridges and setae. There are two series of coeca with an anterior one located behind stomach consisting of thirty elongate, slender unbranched appendages which is placed in a backward position and a second coeca present in abdominal region, consisting of two small dorsal unpaired coeca. There are maxillary glands present, which is possibly a primitive feature. Other glands include the Maxilla excretory glands, which are large, coiled tubes ending in an expanded sac and opening at the proximal margin of the maxilla. Antennary glands are absent. The nerve cord consists of eight free thoracic ganglia and six abdominal ganglia corresponding to each of the body segments

Reproductive System

The sexes are separate. The male *Anaspides* testes are paired thin tubes that extend from the fifth thoracomere to the final pleomere. The vas deferens is directed forward to the gonopore on the eighth sternite (Smith 1909a, Siewing 1959). In Allanaspides the tubes fuse and lead into a single medial gonopore (Swain pers. comm, 1987). Spermatozoa are filiform, with globular heads and an elongate flagellum. A structure shared with Mysidacea. The most prominent feature of the spermatozoa inn *Anaspides* is the highly developed acrosome, which is similar to the Pericarida and Eucarida however, differs by possessing a long, subacrosomal filament that is not in contact with the nucleus at any time during development (Jespersen 1983).

The vas deferens in *Paranaspides* differs from *Anaspides* by having one tube that passes into a blind ended lobe while the other end forms a narrow efferent duct. The duct then coils close to its origin and forming a wide tube directed back to the eighth thoracic segment. The tube is then directed back to the third abdominal segment and bends dorsally forward to the eighth thoracic segment again (Nicholls & Spargo 1932). This recurrent loop is also found in *Micraspides* (Nicholls & Spargo 1932).

The female reproductive system consists of ovary and oviducts. The ovaries (like the testes) are paired tubes that extend the length of the body (Smith 1909a, Siewing 1959). The oviducts open on the medial surface of the thoracopod coxa of thoracopod six. Just distal to the aperture, the coxa bears a setose coxal lobe, that is directed anteriorly and similar lobes are present on the coxa of thoracopods five and six. The male ducts open, not on the coxopodites but on the sternum of the eighth or last thoracic somite, by oblique slits converging anteriorly. The terminal part of the vas deferens is enlarged and apparently glandular. The spermatheca was first discovered in *Koonunga* by Sayce (1908) and described in more detail by Calman (1908). It is a rounded prominence, directed forward, with a wide transverse slit at the apex. This slit opens into a blind sack with thick walls, on each side of which at the proximal margin appears to be a gland opening into a cavity by a short duct. The statement by Thomson 1893 that the "genital opening of the female on the sternum in front of the last pair of thoracic leg" was incorrect and later corrected by Calman 1908 by saying that “the openings of the genital apertures in the female on the sixth and in the male on the eigth thoracic appendage (Swain & Reid, 1983). Development of eggs is temperature dependent (Hickman 1937, Swain & Reid 1983).

The ovaries of *Allanaspides* contain 10-12 eggs that are four times larger than the oviducts. The oviducts are flexible allowing for the passage of the eggs. They then pass out the genital aperture, which does not stretch and regain their initial shape (Swain, R pers.comm 1987). The larval embryology and the level of parental investment in the Anaspididae is considered by Watling (1983) and Hickman (1937) to be the most primitive behaviour within the Eumalacostraca i.e. the deposition of eggs onto the substrate where they hatch resembling the adults.

Physiology and Ecology

The Anaspidacea are almost entirely restricted to cool to cold freshwater environments across their distribution. Swain & Reid (1983) found that *Anaspides sp.* exist in locations that freeze in winter and can be collected in streams and lakes below the ice but that temperature greater than 15 °C caused significant temperature stress in the small alpine streams.

Locomotion and Dispersal

The locomotion in Anaspidacea was first examined by Manton (1930) from live observations of *Anaspides* and *Paranaspides*. Later studies included Hessler (1983) and Schram (1984) in which they also included

Allanaspides and *Micraspides*. *Anaspides* and *Paranaspides* are able to walk on the substrates as well as on aquatic vegetation using all eighth thoracopods i.e. the ambulatory thoracic endopodites. The endopodites consist of elongate, cylindrical segments and a terminal segment, the dactylus that bears one to several enlarged spines or claws. The Anaspididae are the best swimmers of the group. Paranaspides swims by combining its thoracopods and pleopods (). The exopodites of all body segments in *Anaspides* are plumose with a flattened central shaft and lateral setose setae that form a fan structure. These appendages are in constant motion in order to ventilate the respiratory epipodites.

The only published account on the dispersal characteristics of the Anaspidacea was by Manton (1930), and Williams (1965) who reported that *Anaspides* has the tendency to move against the current and was capable of leaving and remaining out of water for some time. This was confirmed by Swain & Reid (1983) with laboratory test showing that *Anaspides tasmaniae* can remain out of water for several days in low temperatures and high humidity. Serov (1988) found that large specimens were observed on several occasions at night to climb upwards along the peripheral margins of rapids moving from one pool to another. All animals were large females ranging size from 35-40mm. This behaviour and their environmental tolerances suggest that overland travel is possible given the right conditions. Dispersal by downstream drift has been reported for two species of Anaspididae including: *Anaspides tasmaniae* (Serov 1988) where they entered the drift after sunset; and *Micraspides* collected from drift nets in the lower part of the King River near Zeehan by the Inland Fisheries Commission, King River Study in 1986 (unpublished data).

Feeding Habits

Although predominantly detritivores, predatory behaviour has been reported for *Anaspides* and *Paranaspide*s where they feed on other invertebrates including worms, tadpoles, insects such as Leptoceridae Trichoptera (Manton 1930) and even cannibalism of smaller animals (Serov 1988) although *Anaspides* is predominantly a detritus feeder consuming plant debris, algal filaments and decaying animal matter. Swain & Reid, (1983) observed a 30mm female attack and devour a tadpole of similar size. Cannibalistic has been reported in laboratory animals as well as in the gut analysis of large females from the wild (Swain & Reid 1983)

Life cycle

Hickman (1937) conducted the first study examining aspect of the life cycle of *Anaspides tasmaniae* on specimens from the Newtown Rivulet on Mount Wellington however, his focus was predominantly on the embryology and juvenile development post hatching up to 20mm. His findings concluded that the larvae lack a free-swimming larval stage and resemble the adults except for fewer limbs. The eggs of Anaspides are 1mm in diameter and pale purple in colour. At hatching the juveniles are 3mm in length (Hickman 1937, Serov 1988).

Once the spermatophores are in place spermatozoa pass into the spermatheca and the spermatophores drop off (Hickman 1937). Egg laying commences with the eggs being passed out the oviduct and are probably fertilized as they pass out. It is possible that they are directed and/or supported by the setae on the medial margin of the coxae of thoracopods 6-8 which appears to be a sexual character of females.

Eggs are laid singly on moss, bark in the stream and adhere to the surface via a sticky coating. Egg-laying goes on throughout the year but is most active during October and November. Hickman's (1937) found in his study of Anaspides tasmaniae eggs laid in spring and summer hatch in 32-35 weeks (8-9 months) however, eggs laid in autumn and winter would develop to the gastrulation stage and go into a dormant state until October after which development would continue until hatching. This would extend the development period up to 60 weeks. This dormancy or diapause phase was linked to seasonal temperature fluctuations (Serov 1988).

Williams (1965b) analysed a small sample of 124 animals from a small stream in the Central Plateau and found there were four size classes (8, 23, 31, and 1 female at 38mm) with no difference between the sexes. Williams (1965b) suggested that *Anaspides* lived for 3-4 years and that females may live longer than males. Swain & Reid (1983) concluded that the Mt Field species lives for 4-4.5 years, whereas Serov (1988) found that *Anaspides* in low altitude streams on Mount Wellington may live up to seven years for females and 5-6 years for males and reproduce 6-8 times in a life span. Serov (1988) also reported that egg numbers increased per animal from 50 eggs for a young females up to 100 eggs for large females over 35mm in length. Two juvenile modes and four adult modes were identified. The juveniles modes includes recently hatched animals of 5mm and the second mode of 8-14mm. Adult modes were in males 16-19mm, 20-23mm, 24-27mm, and >28mm. Females grow at a faster rate with the modes including 17-20mm, 21-27mm, 28-31mm, and >34mm. Female to male ratio is about 1:1 with the females reaching a larger size and longevity to the males (Swain & Reid 1983, Serov 1988).

The life cycle of a closely related genus, *Paranaspides lacustris* differs from *A.tasmaniae* with hatching taking place between June and September and egg laying during October to November (Hickman 1937). Eggs hatch after 6-8 months and the hatchling is approximately 2mm in length. The sexes can be distinguished at 7-8mm and the life span is approximately 18 months (Fulton 1982) with one breeding period per year and maximum length of 25mm. *Allanaspides helonomus* has an earlier hatching period occurring from March to May (Swain, per. comm1987, Driessen. & Mallick 2007). This species reaches a maximum length of 13mm and has a life span of 13-15 months. *A.hickmani* has a maximum length of 11mm.

In the family Koonungidae, Zeidler (1985) noted males are considered sexually mature when the development of sexual organs i.e. Pleopods 1 and 2 are complete. He also stated that development of the sexual organs of the male Koonunga is complete when the coupling hooks of Pleopod 1 are developed and the Pleopod 2 apex has a concave depression.

Breeding Habits

The only study of conducted on the breeding and embryology of the Anaspidacea was that of Hickman (1937) in which he examined *Anaspides tasmaniae* from Mount Wellington, Hobart. The only recorded observations of Anaspidacea mating were described by Smith (1909) for *Anaspides tasmaniae* and McConnell (1987) for *Allanaspides helonomus*. The process of breeding involves the male anaspidaceans depositing two large spermatophores in the spermatheca of the female via a transfer from the vas deferens through the petasma. Mature males of *Anaspides* can be identified by white coloured swelling of the lateral side of the eighth thoracomere (Serov 1988).

Habitats

The anaspidaceans have always been indicators of cool, temperate, permanently wet habitats as they have no stage in their life cycle that can tolerate desiccation although the eggs of *Anaspides* do have a temperature dependent diapause function to stop development during the winter periods however, they still need to permanently wet (Hickman 1937, Swain & Read, 1983). Tasmania, in particular, has the most diverse syncarid fauna in the world, that inhabit a plethora of environments from highland tarns and lakes, to rivers, swamps, caves and even the interstitial environment of underground waters and springs.

The species found in NSW have no surface species but do occupy a number of caves and aquifers and form a species complex throughout central, eastern and northern NSW. These species belong to two different families of the Syncarida. The first and most broadly distributed group is the family Psammaspididae. This family occurs from northern NSW through the Central West and down to the southern East coast. They inhabit a range of cave types from the active streamways of Jenolan, such as *P. jenolanensis* that was found in Imperial Cave, to still water, impounded karsts such as Wellington Caves. The second group is the new family Raptornungidae. This family occupies the same subterranean environments as the Psammaspididae however, occupy the adjacent northern region that encompasses the mid north coast of New South Wales, up to almost the Queensland border (Serov 2002).

Threats and Conservation Value

Currently only four species of Anaspidacea are listed by the World Conservation Union (IUCN) (Wells, *et al*. 1983) as Vulnerable, or facing a high rate of extinction in the wild. These include the Tasmanian *Allanaspides helonomus*, Hickman's Pygmy Mountain shrimp (*Allanaspides hickmani*), the Great Lake Shrimp (*Paranaspides lacustris*) within the Family Anaspididae, and *Eucrenonaspides oinotheke* within the family Psammaspididae. See Table below.

Anaspidacea Species	**Conservation Status**
Allanaspides helonomus (Tasmanian Anaspid Crustacean)	Vulnerable D2 ver 2.3
Allanaspides hickmani (Hickman's Pygmy Mountain Shrimp)	Vulnerable D2 ver 2.3
Paranaspides lacustris (Great Lake Shrimp)	Vulnerable D2 ver 2.3
Eucrenonaspides oinetheke	Vulnerable D2 ver 2.3

Table 1.4 Anaspidacea species conservation status listed within the IUCN 2013. IUCN Red List of Threatened Species, Version 2013.1. Downloaded on 22 August 2013.

The main threats that have been previously documented include predation by introduced species (Williams 1965, Yen & Butcher 1997), fire (Howitz 1990) and impacts to the water source either through the flooding of habitat through the creation of dams (Yen & Butcher 1997) or reductions in lake or groundwater through either extended drought or active water extraction.

One of the main threats to the surface water species such as *Anaspides* and *Paranaspides* is predation by introduced trout. There is good correlation between the presence of trout and the absence of Anaspides (Williams 1965; Lake & Knott 1972; Knott, *et al*. 1978, Yen & Butcher 1997), suggesting that the shrimp exists where predation by trout is absent or not heavy. *Paranaspides lacustris* is a common component of the food of trout (Evans 1937-39) in Great Lake. It is not known whether predatory pressure in this species by introduced species could lead to its elimination or reduction in numbers however, there have been at least two periods of apparently reduced population size of *Paranaspides* (1920s and 30s and after 1966) that followed the artificial elevations of the water level of the lake. These large and persistent fluctuations of water levels in the lakes are likely to result in the reduction in the areal extent or elimination of the weed beds which support *Paranaspides lacustris* (Fulton, pers comm.). Lake and Coleman (1974) called for a reserve to be created for the *Paranaspides* in a lagoon which is not subject to water level fluctuations.

There have been two main threats to the species of *Allanaspides* within the Buttongrass peatland wetland communities of the Lake Pedder/Lake Gordon catchments. These have been fire and flood (Howitz 1990, Yen & Butcher 1997). The peat swamps they occupy and the short lifespan of these species put them at

risk from any process which impacts the water levels or habitat outside the natural ranges such as a sustained drought, fires in peat, has the potential to eliminate populations.

The firing of buttongrass areas occurs frequently in south western Tasmania. This has resulted in the elimination of peats from large areas and the erosion of the underlying material (Pemberton 1988). Such fires can result in total habitat loss. Currently little damage has been reported within the ranges of either of the Allanaspides species, and their habitats appear to be dependent on the presence of a shallow groundwater watertable in the peat, the soil may only rarely be combustible.

The flooding of buttongrass plains has already significantly reduced the range of species of *Allanaspides* (Wells, *et al.* 1983, Yen & Butcher 1997) since individuals are not likely to have survived in the lake environment due to predation of native ad introduced fishes as well as the loss of their principle habitat, the Pholeteros or water within the burrows of the Land Crayfish, *Engaeus*. Further rises in the water levels of the lakes, although not planned, will further reduce the available habitat. The impact of flooding of the Lake Pedder Catchment may have resulted in the extinction of the Lake Pedder *Anaspides* as it has not been recorded in the lake since the filling of the lake in 1972.

Systematics

The Position of the Syncarida

(Figures 1. 2-1.6)

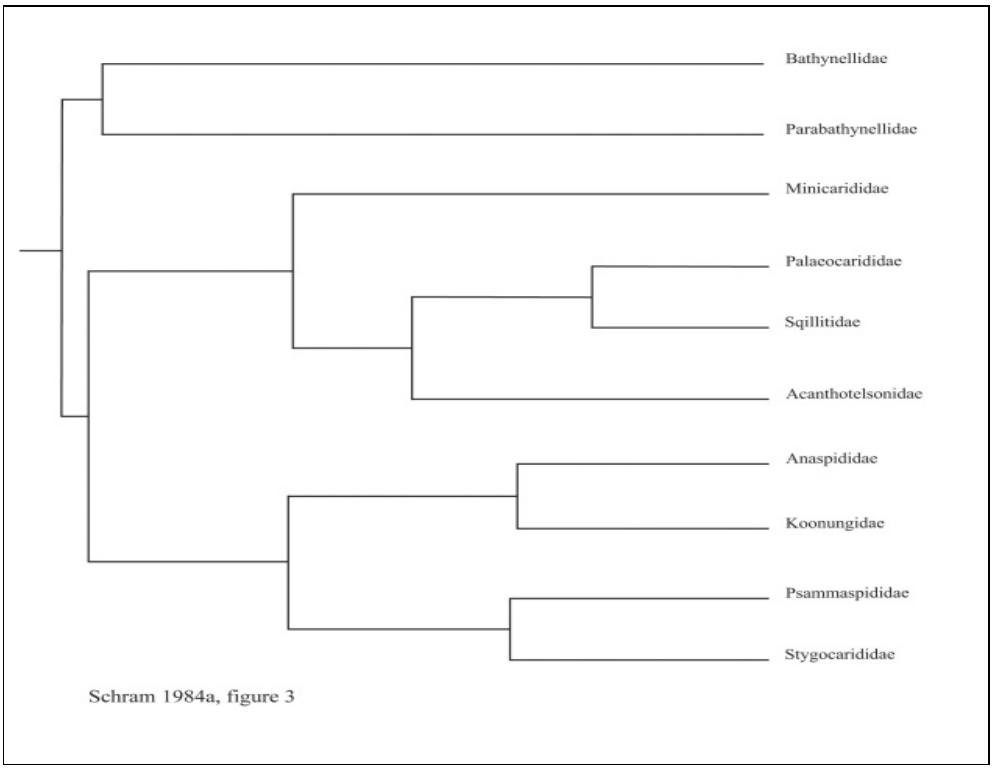
The position of the Syncarida within the Subclass Eumalacastraca Grobben 1892 and the Class Malacostraca Latreille 1802 is an important starting point for understanding the processes that have led to the current position of the Anaspidacea within the Syncarida as well as the current classification arrangement of taxa within the Anaspidacea. The early studies of the Anaspidacea (based on gross morphological features) (Schram 1984a, Figure 1.2 and 1.3) first demonstrated that the Syncarida were a primitive group within the Crustacea due to the possession of a number of plesiomorphic features. The classification of the Syncarida and its two extant orders (Anaspidacea and Bathynellacea) were originally accepted as a monophyly (Schram 1984a, Richter & Scholtz 2001, Lopretto & Morrone 1998, Martin & Davis 2001). The position of the Syncarida and the Anaspidacea within the Crustacea changed frequently over the last 130 years as the characters used were refined and more taxa were added. In most of the phylogenetic studies featuring the Syncarida and the Anaspidacea the features used were that *Anaspides tasmaniae*. For this reason all earlier studies based on morphology presented a rather simplified phylogeny which did not take into account of the diversity of features that we now know exists in the Suborder today.

Thomson was the first to include the Anaspidacea into a classification in 1894 following the discovery of *Anaspides tasmaniae*. In this classification the Anaspidacea was grouped within the Schizopoda with the

Mysidacea and the Euphausiacea. Calman in 1904 abolished the Schizopoda and joined the Bathynellacea with the Anaspidacea to form the Syncarida. Siewing (1959) placed the Syncarida as basal to all crustacea except for the Stomatopoda and Leptostraca. This arrangement continued until 1989 when Walting elevated the Syncarida above the Isopoda and Amphipoda and below the Decapoda. In 1999, Lane and Schram once again considered the Syncarida to be highly derived in terms of the Crustacea by placing it within the Malacostraca. Within the Malacostraca they considered the Syncarida to be the most plesiomorphic group, however this time they separated the Bathynellacea as the basal lineage followed by the Anaspidacea (Figure 1.3b).

Schram (1984) was one of the first to present an analysis of all Syncarida families including the fossil syncarids in the Palaeocaridacea. In this paper he presented a number of alternative hypotheses. The tradition approach (Figure 1.2 a) presented the Bathynellacea as the most plesiomorphic clade based predominantly on the presence of the caudal furca, whereas the Palaeocaridacea and Anaspidacea is delineated by the lack of a furca and the first thoracopod was modified. He also presented three alternate phylogenies where the first dichotomy was based on the degree of fusion of the first thoracomere with the cephalon. In this arrangement the Bathynellacea become a sister group with the Minicaridae. This analysis also joined the Anaspididae with the Koonungidae and the Psammaspididae with the Stygocarididae. The last two alternative cladograms presented by Schram (1984) joined the Bathynellacea with the Palaeocaridacea and separated the Anaspidacea primarily based again on the degree of fusion of the first thoracomere while still retaining the earlier two grouping of the Anaspidacea (Figures 1.2 b and 1.3a).

a.



b.

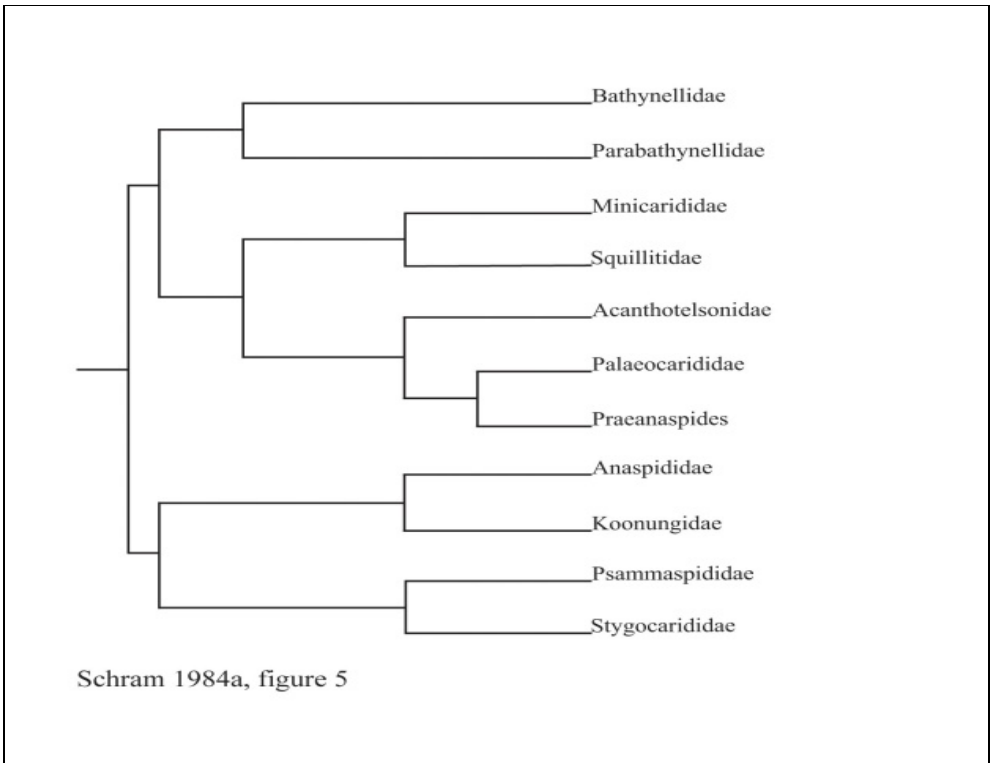
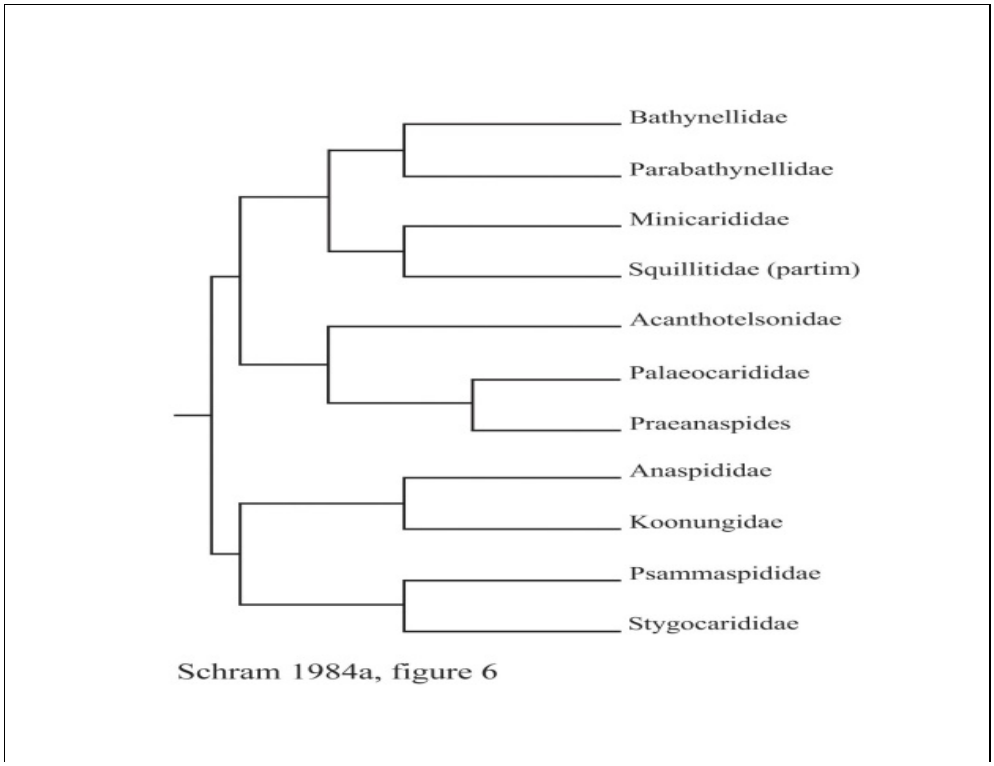


Figure 1.2 a & b. Phylogeny of the Syncarida based on morphological characters. a. Traditional view of the Syncarida (Modified from Figure 3, Schram 1984). b. An alternative phylogeny reversing the polarity of the loss of fusion of the cephalon with the thoracomere 1. (Modified from Figure 5, Schram 1984).

a.



b.

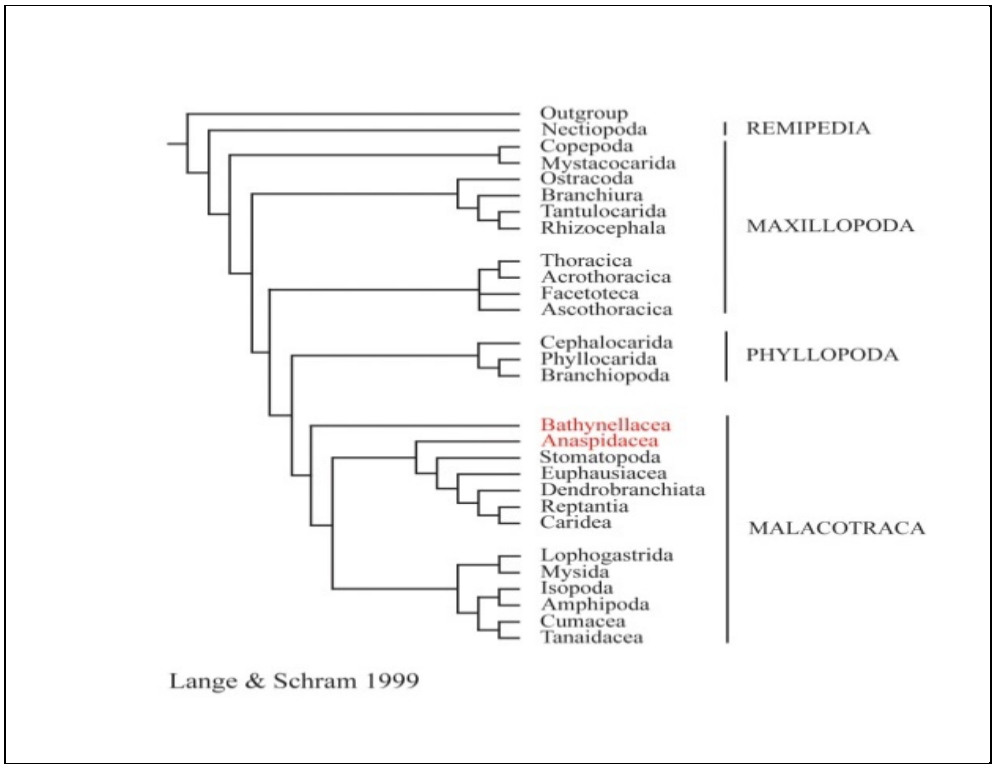
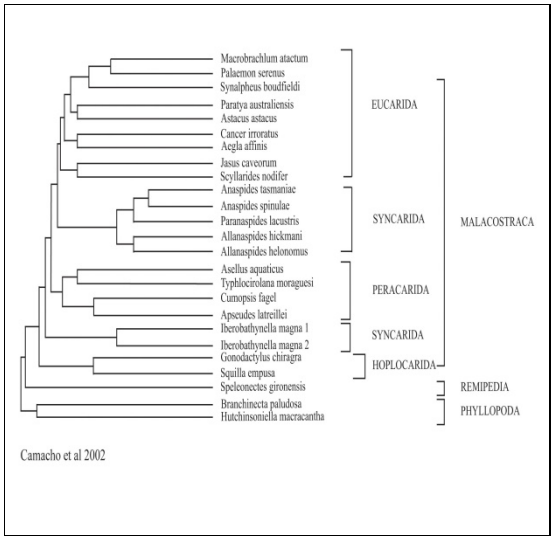


Figure 1.3 a. Another alternative phylogeny reversing the polarity of the loss of fusion of the cephalon with the thoracomere 1. (Modified from Figure 6, Schram 1984). b. Phylogeny of the Crustacea based on morphological characters based only on extant taxa (Modified from Lange and Schram 1999, Schram & Hof 1998, and Coineau & Camacho 2013).

Throughout the 1990s and early 2000s new phylogenetic methods were developed (Coineau & Camacho 2013) for morphological analysis of phylogeny (e.g. Martin & Davis 2001, Brusca &Brusca 2003). The Anaspidacea and Bathynellacea were variably considered with the common consensus being that the Bathynellacea would appear in a basal position such as Lange and Schram (1999) (see Figure 1.3 b) whereas the Anaspidacea would be in a more derived position although its position in relation to the Bathynellacea was variable. In the early 2000s the first molecular based phylogenetic analyses were being conducted such as Camacho *et al.* 2002 using 16s rRNA) (Figure 1.4a) which confirmed the basal position of the Bathynellacea as a sister group to the other Eumalacostraca and reinforcing the concept that the Syncarida is a paraphyletic grouping i.e. the Bathynellacea should not be included with the Anaspidacea. Jenner (2009) performed a more comprehensive analyses combining both morphology and a range of genetic markers including 18S rRNA, 28S rRNA, COI, 16S rRNA also demonstrated the separation between the two orders (Figure 1.4b). More recent studies by Coineau & Camacho 2013) suggest that the Syncarida are monophyletic (Figure 1.5).

The use of fossils combined with extant taxa of the Syncarida has only been conducted in two studies. These included Schminke (1975) and Schram (1984). Coineau & Camacho (2013) presented the most probable phylogeny (Figure 1.5) which indicates that the Bathynellacea forms the ancestral or basal clad to the sister groups, the Palaeocaridacea and Anaspidacea. It also interprets the position of the Anaspidacea as being derived and evolving at a much later stage to the Bathynellacea and the Palaeocaridacea Mesozoic compared with the Devonian) based primarily on the fusion of thoracomere 1 with the cephalon.

a.



b.

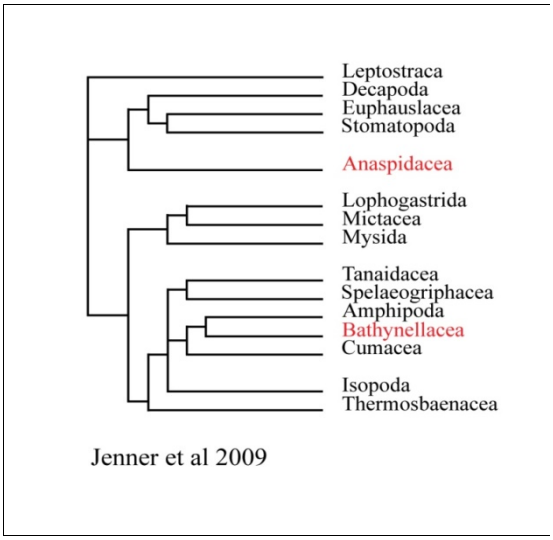


Figure 1.4 a & b. Phylogeny of the Eumalacostraca based on genetic characters. a. Analysis based on mt16S sRNA (Modified from Camacho et al. 2002 and Coineau & Camacho 2013). b. Analysis based on a combination of 18S rRNA, 28S rRNA, COI, 16S rRNA and morphological characters. (Modified from Jenner et al. 2009 and Coineau & Camacho 2013).

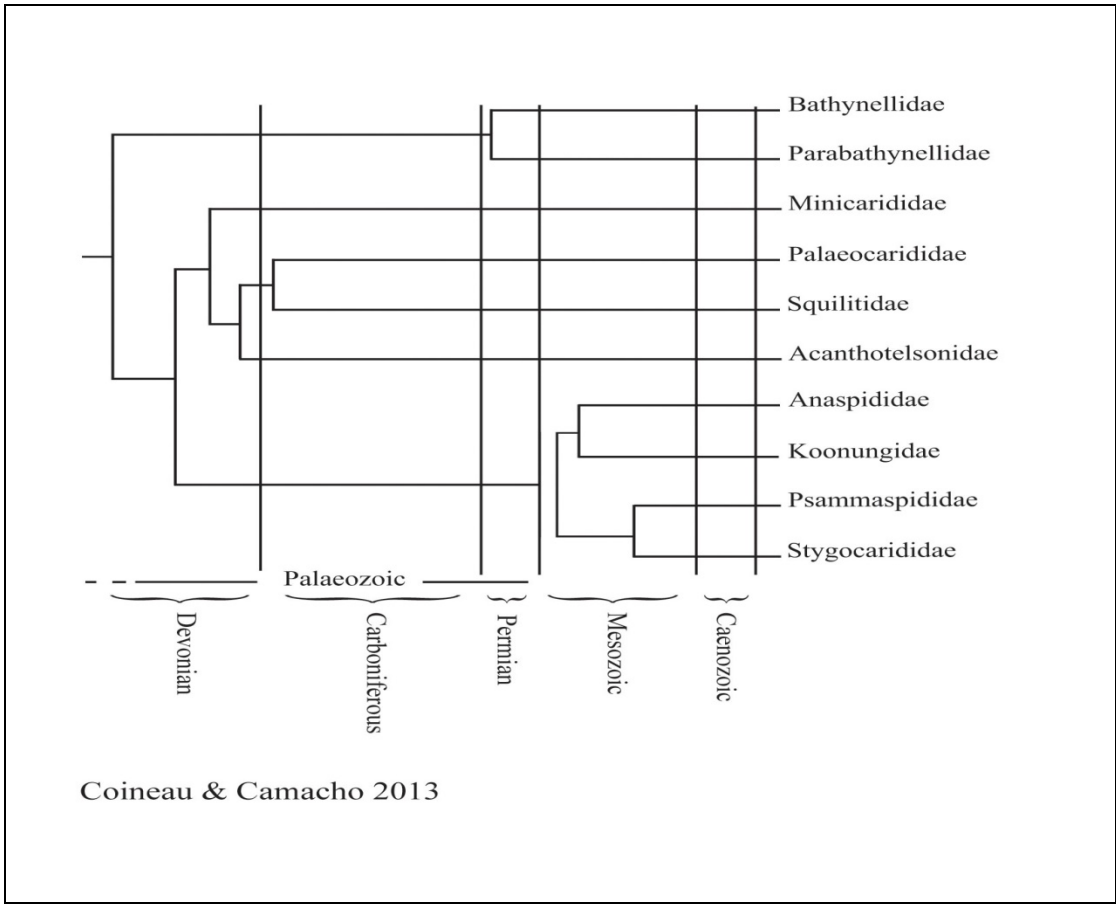


Figure 1.5. Phylogeny of the Syncarida based on synapomorphic morphological character states. (Modified from Coineau & Camacho 2013, Schminke 1975 and Schram 1984).

Systematics within the Anaspidacea

The Order Anaspidacea is one of three orders within the Superorder Syncarida, which is a prominent group within the Class Malacostraca and subclass Eumalacostraca and a sister group to the Pericarida and Eucarida. Although the Syncarida was not erected until 1885, the history of the group began in 1947 by the description of the Permian fossil *Gamponyx fimbriatus* by Jordan (1947) which was later renamed by Schram in 1984 to *Uronectes fimbriatus.* Roemer (1856 in Schram 1984) initially placed the species within the Stomatopoda. In 1865, two other Carboniferous fossils, *Acanthotelson stimpsoni* and *Palaeocaris typus* (that were also later to be identified as Syncarida) were placed within the Isopoda by Meek and Worthen (1865) It was then not until 1885 until Packard recognised the separate status of *Acanthotelson stimpsoni* and proposed the taxon Syncarida.

The first living anaspidacean to be described was *Anaspides tasmaniae* Thomson 1894. *Anaspides tasmaniae* was originally described by Thomson (1893, 1894) from specimens collected on Mt Wellington, adjacent to Tasmania's capital city, Hobart. This species was initially designated to the genus *Anaspis*

Thomson 1883 but a year later (1894) Thomson reassigned it to the Genus *Anaspides*. Although the exact reason for this change was not discussed, the name *Anaspis* had previously been assigned to a beetle genus described by Geoffroy in 1762. The etymology of the name was not given however, *Anaspides* or *Anaspida* is the Latin translation derived from the Greek word anaspis, ἄνασπις. This is interpreted to mean *'an - aspis* or no shield' or carapace. This refers to the original generic Greek word Aspis or shield which historically refers to the characteristic shield carried by the ancient Greek Hoplites.

In his first description of the species Thomson initially aligned it with the Schizopoda, a Division of Malacostraca that was superseded by the orders Mysidacea and Euphausiacea. The division Schizopoda included shrimplike Thoracostraca in which each of the thoracic legs has a long fringed upper branch (exopodite) for swimming. *A. tasmaniae* later became the subject of great curiosity among zoologists following Calman's (1896) recognition that it was, in fact, related to another group of malacostracans that were known only from fossils found in Europe and North America belonging to the Order SYNCARIDA Packard 1885. In 1904 and 1909 Calman outlined the first modern classification of Syncarida, with only a minor reference being made to the possible phylogentic relationships (Schram 1984).

The Order Anaspidacea was first erected by Calman in 1904 to encapsulate the genus *Anaspides* as a separate group and to unite it with the northern hemisphere fossil species in the new Superorder Syncarida Calman, 1896. Sayce (1908) later redefined the Order by providing the initial description. In 1959 Siewing presented a classification for the Syncarida that divided it into three Orders: the Grampsonychidae Packard 1885; the Bathynellacea Chappuis 1915, and the Anaspidacea Calman 1904. The Order Grampsonychidae was characterised by having seven abdominal somites and a statocyst associated with the uropod. Brooks (1962) however showed that neither the seventh abdominal segment nor the uropodal statocyst existed.

Siewing (1959) defined the Anaspidacea as having six abdominal somites, antennules with statocycts and the females with a spermatheca on thoracomere 8. He included within this Order the Anaspididae Thomson 1893, the Koonungidae Sayce, 1908 and a new Family, the Palaeocarididae in which the members had seven free thoracic somites. He included this fossil family in an attempt to link the fossil Syncarida with the extant Syncarida. Similar attempts by other authors such as Vandenburghe, 1960 and Rolfe, 1962 also attempted to combine the two groups however, this only created systematic confusion as their classifications were based on the misconceptions of early palaeontologists (Brooks 1962). In 1962, Brooks removed the Family Palaeocarididae from the Anaspidacea and raised it to the Ordinal status, on the grounds that it's' members possessed eight thoracic segments in contrast to the seven in the Anaspidacea.

Brooks (1962) followed by classifying the extant Syncarida into two Orders: the Anaspidacea; and the Bathynellacea. In his classification of the Anaspidacea he included the fossils, *Anaspidites antiquus* as a new genus within the Anaspididae and the species *Clarkecaris brasilicus* as a new family, Clarkecarididae.

Both of these species were later subverted by Schram (1984) into the Family Incertae sedis within the Order Palaeocaridacea due to his uncertainty about this systematic position.

The Order Stygocaridacea was erected by Noodt in 1965b by elevating the family Stygocarididae that was created by Noodt in 1963a to accommodate the genera *Stygocaris* and *Parastygocaris*. Schminke (1980) presented an examination of the Stygocarididae with the addition of new genera and species from New Zealand. The Order Stygocaridacea was later subverted to a subordinal status to Stygocaridinea by Knott & Lake in 1980 and placed within the Order Anaspidacea. The concept that the stygocarids should not be an order was also suggested by Schminke in 1975 where he treated Stygocarididae as belonging to the Anaspidacea although he did not qualify the reasoning behind the placement. The Stygocarididae are currently known from South America, Australia and New Zealand. Only one species has been recorded from Australia from one site in northern Victoria.

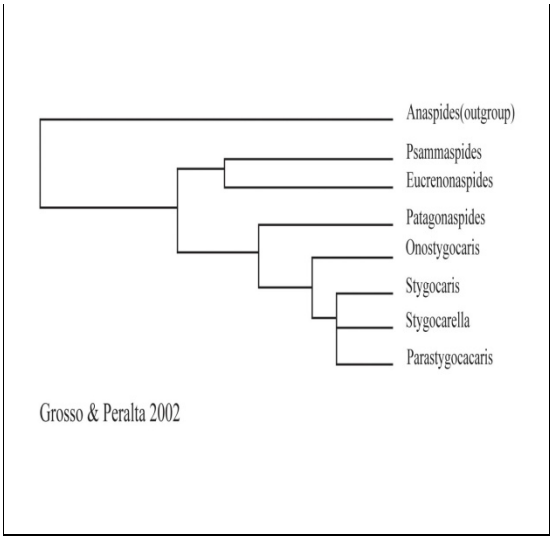


Figure 1.6. Phylogeny of the Stygocarididae based on morphological character states (Modified from Grosso & Peralto 2002).

In 2002, Grosso and Peralto presented the first phylogenetic analysis of the Stygocarididae based on morphology in order to determine the possible relationships of the Stygocarididae and the Psammaspididae with the newly discovered Patagonaspididae. In this analysis *Anaspides tasmaniae* was used as the outgroup. The result of the analysis proposed that the Psammaspididae was basal to a monophyletic group including the Patagonaspididae and the Stygocarididae. The Patagonaspididae was also basal to the Stygocarididae.

In 1980 Knott and Lake proposed a new classification for the Anaspidacea which included the following characters in order to incorporate the genus *Eucrenonaspides*: the absence of eyes, an incisor accessory process on the mandible; endopodites generally with uniform morphology; exopodites usually well

developed, sometimes absent; epipodites occurring on first five or six thoracopods; pleopods natatory (i.e. developed) reduced or absent; and no appendix interna. They however, did not include the character "female with spermatheca".

An interesting note is that Knott and Lake (1980) added the processus incisivus accessorius to the diagnosis of the Anaspidacea in order to make it more compliant with *Eucrenonaspides* and yet neither *Eucrenonaspides* nor any of the other families possess this structure. The structure within the Anaspididae is a fine setal row associated with the molar process rather than with the incisor process. The only family that may be regarded as possessing this feature is the Stygocarididae which have a small number of brush-like penicillate setae on the ridge between the incisor and the molar processes. Therefore, this character does not belong in the ordinal diagnosis. The delineation of the suborders by Knott & Lake (1980) however, are useful in grouping the Psammaspididae with the Stygocarididae however, the grouping of the Koonungidae with the Anaspididae was based on the misinterpretation of characters (also misinterpreted in Kutschera *et al*. 2012), ignoring more diagnostically significant characters. This is further discussed below.

In 1907, Sayce added a second family, the Koonungidae to the order and a new genus and species *Koonunga cursor* Sayce 1907. The etymology of the name came from an Aboriginal word for the water course from which it was discovered. It was this time collected from wetlands in Melbourne, Victoria in mainland Australia. It would take another 30 years before Nicholls discovered and described another Koonungidae, *Micraspides calmani* from seeps and small streams on the west coast of Tasmania in 1931. In 1985, 54 years later, the second species of *Koonunga*, *Koonunga crenarum* Zeidler 1985 was discovered in caves along the southern Victorian and South Australia border.

A second species of *Anaspides* was discovered in 1965 on the benthos at 3m depth, in the cold waters within Lake St Clair in the Central Plateau of Tasmania. It was described as *Anaspides spinulae* Williams 1965b on the basis of several distinct, but variable, morphological features and its probable reproductive isolation from nearby populations of *A. tasmaniae* (Williams 1965b; Schminke 1982). *Anaspides* with some or all of the distinguishing morphological features of *A. spinulae* have more recently been found in several locations on the Central Plateau other than Lake St Clair (O'Brien 1990).

The second genus of the Anaspididae, *Paranaspides lacustris* Smith 1908 was discovered and described by Smith in 1908 from Great Lake and nearby lagoons to the east of Lake St Clair. This is the only pelagic member of the Anaspidacea. The last described members and fourth genus of Anaspididae, *Allanaspides*, was found in 1970 to be part of the community living in the water filled burrows (pholeteros) constructed by *Parasticoides* crayfish in south-west Tasmania (Swain *et al*. 1970, 1971; Schminke 1982). *Allanaspides helonomus* Swain, 1970 occurred in burrows around the edges of now submerged Lake Pedder whereas

Allanaspides hickmani Swain 1971 is only currently found within a tiny area on the buttongrass plains to the south of Lake Gordon.

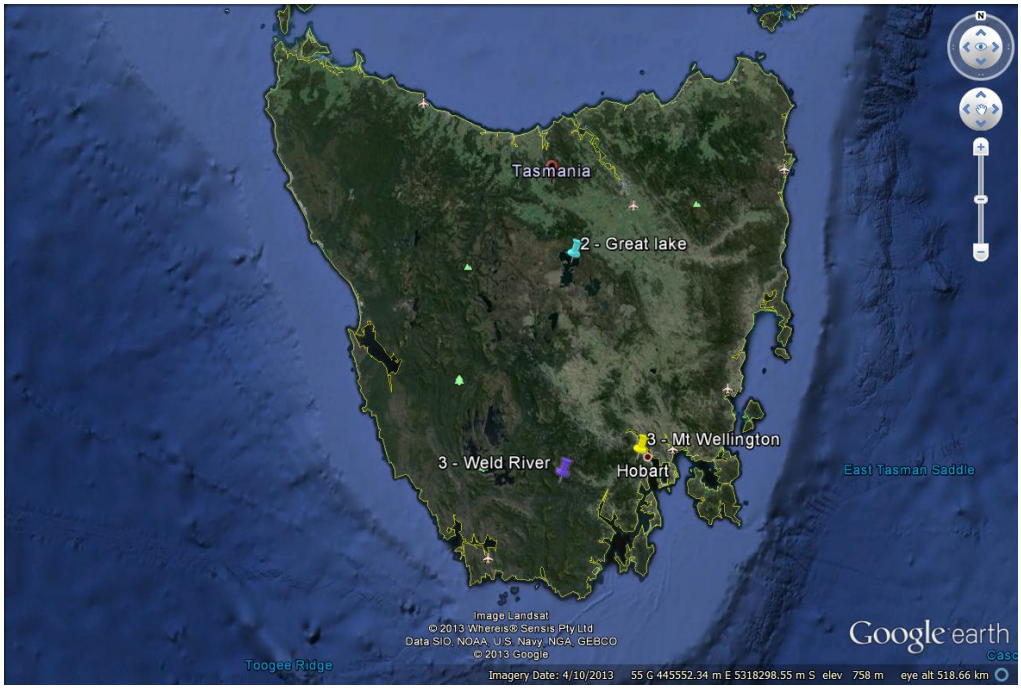
The family Psammaspididae was also included within the Stygocaridinea by Knott & Lake (1980) however this family is being treated separately for the time being.

The next family, genus and species to the added to the list of Anaspidacea is the monospecific Family Patagonaspididae and the species *Patagonaspides sandroruffoi* Grosso &Peralto 2002, which was collected and described in 2002 from a well in Allen, Rio Negro, Argentina, Patagonia, South America by Grosso and Peralto.

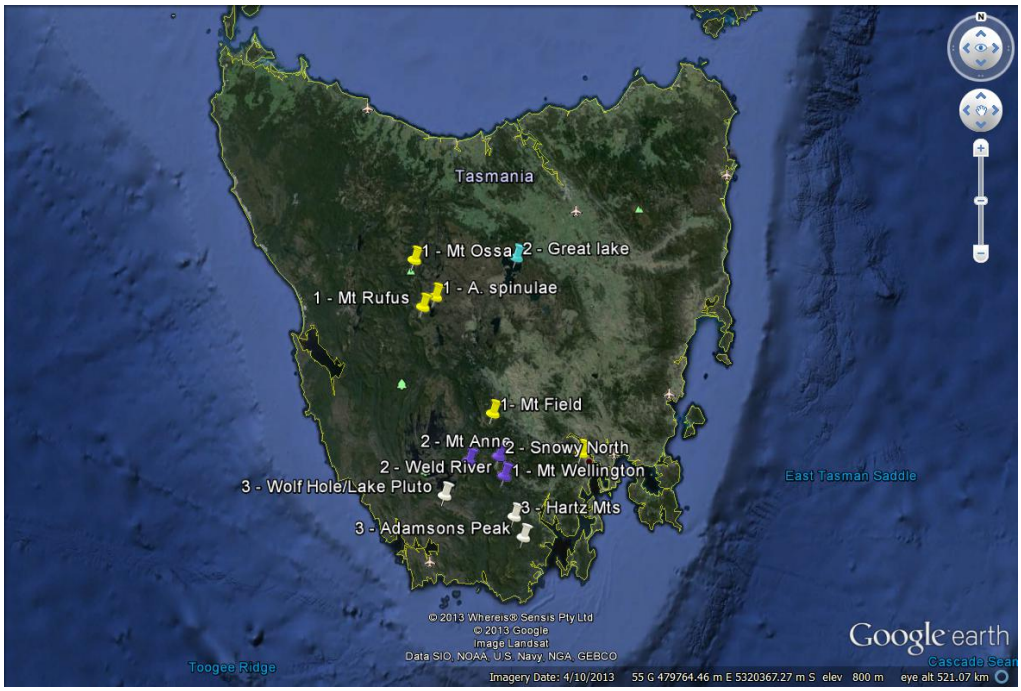
The last family was added in a briefly described by Serov 2002 and given a tentative name of Family A. This family is designated as the Raptornungidae N. Fam in this thesis and fully described (see Chapter 2.4). It occurs in the NE region of NSW Australia from the hypogean and hyporheic zones of easterly flowing rivers and across the Great Dividing Range into the aquifers and hyporheic zones of the rivers and flood plains of the upper Murray-Darling River system.

The description of species of *Anaspides* has followed a slow path of separation. *Anaspides* was originally described from Mt Wellington in 1893 as a single species, *Anaspides tasmaniae*. It was accepted for many years that this was a single widespread species covering most of the highland areas of Tasmania although some researchers had indicated a belief that this species could be separated into more species (Swain, pers comm.). In 1965 Williams discovered a second species, *Anaspides spinulae* in Great Lake.

In order to examine the proposal that there were more species across Tasmania, Andrew (1999) (Map 2.2) was the first to use electrophoretic techniques to study an array of populations of *Anaspides* from across the island including *Paranaspides lacustris* as the outgroup for the analysis. The results of the study concluded that three clades of Anaspides were identified. This included one) Central Highlands populations of *Anaspides* including *A. spinulae, 2)* a southern population of *Anaspides* that were found to be highly differentiated from both the Central Highlands group and each other, indicating a far more complex array of species and maybe even genera along the southern margin of Tasmania, with one population from the Hartz Mountains being similar genetically to the outgroup, *Paranaspides lacustris,* and finally 3) the separate Mt. Wellington type locality for *A. tasmaniae*.



Map 1.3. Species separation of *Anaspides* as described in Andrews (1999). (Google Earth 2013).



Map 1.4. Species separation of *Anaspides* as described in Jarman & Elliot (2000). (Google Earth 2013).

In 2000, Jarman & Elliot examined the evolutionary relationships between the five extant species of the Anaspididae by phylogenetic analysis of partial nucleotide sequences of their mitochondrial large subunit ribosomal RNA genes (16S rDNA). This study uncovered similar, though slightly different and more detailed results (Map 1.4). The results once again recognized at least three ‘species’ clades which included:

1) '*Anaspides* Species 1' (Clade E) which represents a Derwent Valley/Central Highlands grouping of the Derwent River Catchment. This group contained separate populations that included *A. tasmaniae* from the type locality (Mt Wellington), a Mt Field population, *A spinulae* from Lake St Clair, Mt Rufus, Mt Ossa, and Sandbanks Tier populations;
2) '*Anaspides* Species 2' (Clade F) included a Snowy North, Weld River and Mt Anne group of the Gordon River Catchment and;
3) '*Anaspides* Species 3' (Clade D) a group of separate southern Huon River Valley including separate populations at Adamson's Peak, Hartz Mt and the Lake Pluto/Wolf Hole Cave.

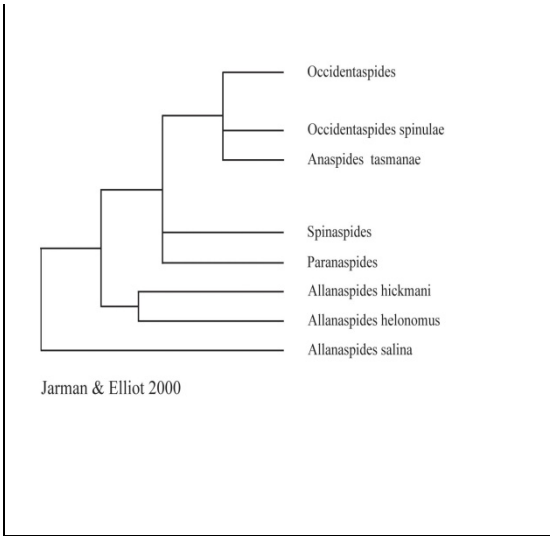


Figure 1.7. Phylogeny of the Anaspididae based on 16S rRNA using the classification of the Anaspididae outlined in Chapter 3.1. (Modified from Jarman & Elliot 2000).

The phylogenetic analyses using 16S rDNA sequences by Jarman and Elliot (2000) estimated that the Anaspididae separated from a common ancestor approximately 55 million years ago with the separation of *Allanaspides* and *Anaspides/ Paranaspides* with further radiations/speciation's occurring at 26, 25 and 12 mya. The next event was the radiation of *Allanaspides* into two species at 26 mya. The separation of *Paranaspides lacustris*, the Derwent Valley *Anaspides* (including *A. tasmaniae*) and Huon Valley *Anaspides* sp. 3 occurred together at _25 Ma. The close time periods of these speciation events during Oligocene/Miocene boundary (26-24 mya) is suggested by Jarman and Elliot, (2000) to indicate a period of significant environmental change. The last estimated separation was between the Central Highlands *Anaspides* sp. 1 and the Snow Mt/Weld River *Anaspides* sp. 2 dated to be approximately 12 mya.

When the species clades identified in the above phylogenetic analysis are replaced with the new genera and species described in this study (Figure 1.7) it demonstrates the possible phylogenetics relationships between the new taxa.

Classification of the Syncarida prior to this study

(Table 1.2)

The current listing of all species of the Anaspidacea and Palaeocaridacea are listed below and organised in their current systematic order. The two families of Bathynellacea have been included for completeness however, as the Order will not be included in this study they are not included in the species list. For the most up to date review of the Bathynellacea see Camacho *et al.* 2006. One very important point that was highlighted by this study was that highly endemic, localised (short range) nature of all anaspidacean species highlighted by consistent morphological differences being detected in specimens as close as 15 kms. Given the very weak dispersal capabilities of this order to move between catchments, subcatchments or even within the same aquifer, this high level of endemicity is to be expected and anticipated, particularly with the more cryptic, subterranean (stygofaunal) families of Anaspidacea.

An examination of the Systematics and Phylogenetics of the Anaspidacea would not be complete, or achieved at all, without an understanding of the speciation, dispersal, distribution, and ecology of its predecessors, the Palaeocaridacea. The Palaeocaridacea is the extinct branch of the Syncarida and are only known through a very long and diverse fossil record that spans over 200 million years from the Carboniferous to the Aptian Epoch of the Lower Cretaceous and spanning the continents of North America, Northern Europe, South America and Australia.

The basic anaspidacean body plan separates this super order from all others. Each family has its own apomorphies and there has been extensive debate on which of the features are plesiomorphic and therefore what is the correct evolutionary pathway (Coineau, & Camacho 2013, Gordon1964, Grosso & Peralta 2002, Hickman 1937, Knott & Lake 1980, Lopretto & Morrone 1998, Schminke 1974a, Schram 1984a.

Current List of Anaspidacea and Palaeocaridacea

Table 1.2. Current taxonomy of the Anaspidacea and Palaeocaridacea.

Superorder SYNCARIDA Packard 1885

Order ANASPIDACEA Calman 1904

Suborder ANASPIDINEA CALMAN 1904

Family ANASPIDIDAE Thomson 1893

Genus *Anaspides* Thomson 1894 (=*Anaspis* Thomson 1893)

Anaspis tasmaniae Thomson 1893

Anaspides spinulae Williams 1965

Genus *Paranaspides* Smith 1908

Paranaspides lacustris Smith 1908

Genus *Allanaspides* Swain, Wilson, Hickman & Ong 1970

Allanaspides helonomus Swain, Wilson, Hickman & Ong 1970

Allanaspides hickmani Swain, Wilson & Ong 1971

*Genus *Anaspidites* Brooks 1962

**Anaspidites antiquus* (Chilton) 1929

*Genus *Koonaspides* Jell & Duncan 1986

**Koonaspides indistinctus* Jell & Duncan 1986

Family KOONUNGIDAE Sayce, 1908

Genus *Koonunga* Sayce 1907

Koonunga crenarum Zeidler 1985

Koonunga cursor Sayce 1907

Genus *Micraspides* Nicholls 1931

Micraspides calmani Nicholls 1931

Suborder STYGOCARIDINEA NOODT 1965

Family STYGOCARIDIDAE Noodt 1963

Genus *Parastygocaris* Noodt 1963

Parastygocaris andina Noodt 1963

Parastygocaris clapsi Grosso & Peralto 1997

Parastygocaris goerssi Noodt 1963

Parastygocaris schminkei Grosso & Peralto 1997

Genus *Oncostygocaris* Schminke 1980

Oncostygocaris patagonica Schminke 1980

Genus *Stygocarella* Schminke 1980

Stygocarella pleotelson Schminke 1980

Genus *Stygocaris* Noodt 1963

Stygocaris giselae Schminke 1980

Stygocaris gomez-millasi Noodt 1963

Stygocaris hugofernandezi Grosso & Peralto 1997

Stygocaris townsendi Morimoto 1977

Family PSAMMASPIDIDAE Schminke 1974

Genus *Eucrenonaspides* Knott & Lake 1980

Eucrenonaspides oinotheke Knott & Lake 1980

Genus *Psammaspides* Schminke 1974

Psammaspides williamsi Schminke 1974

Family PATAGONASPIDIDAE Grosso & Peralto 2002

Genus *Patagonaspides* Grosso & Peralto 2002

Patagonaspides sandroruffoi Grosso & Peralto 2002

Family A Serov 2002

***Order PALAEOCARIDACEA** Brooks 1962

***Family MINICARIDAE** Schram 1984

*****Genus *Minicaris* Schram 1979

******Minicaris brandi* Schram 1979

*****Genus *Erythrogaulus* Schram 1984

******Erythrogaulus carrizoensis* Schram 1984

***Family ACANTHOTELSONIDAE** Meek and Worthen 1865

*****Genus *Acanthotelson* Meek and Worthen 1865

******Acanthotelson stimpsoni* Meek and Worthen 1865

******Acanthotelson kentuckiensis* Schram 1984

*****Genus *Uronectes* Bronn, 1850 (= *Gampsonychus* Burmeister 1855)

******Uronectes fimbriatus* Jordan 1847

******Uronectes kinniensis* Schram & Schram 1979

******Uronectes palatinus Uhl and Raisch 1999(see Uhl 1999)*

*****Genus *Palaeosyncaris* Brooks, 1969

******Palaeosyncaris dakotensis* Brooks, 1969

******Palaeosyncaris micra* Schram 1984

***Family PALAEOCARIDIDAE** Meek and Worthen 1865

*****Genus *Palaeocaris* Meek and Worthen 1865

******Palaeocaris typus* Meek and Worthen 1865

******Palaeocaris secretanae* Schram 1984

******Palaeocaris retractata* Calman 1932

*****Genus *Monicaris* Stamberg 2000

*****_Monicaris rubnicensis_ Stamberg 2000

***Family SQUILLITIDAE** Schram and Schram 1974

*****Genus _Squllites_ Scott 1938

*****_Squillites spinosus_ Scott 1938

*****Genus _Praenaspides_ Woodward 1908

*****_Praenaspides praecursor_ Woodward 1908

*****Genus _Nectotelson_ Brocchi 1880

*****_Nectotelson krejcii_ Brocchi 1880

***Family UNCERTAIN (Incertae sedis) Schram 1984**

*****Genus _Pleurocaris_ Calman 1911a

*****_Pleurocaris annulatus_ Calman 1911a

*****_Pleurocaris juengeri Schöllman 1999_

*****_Genus Spinocaris Uhl 1999_

*****_Spinocaris horribilis Uhl 1999_

*****Genus _Williamocalmania_ Schram 1984

*****_Williamocalmania vandergrachti_ (Pruvost) 1912

*****Genus _Brooksyncaris_ Schram 1984

*****_Brooksyncari canadanensis_ (Brooks) 1969

*****Genus _Palaeorchestia_ Zittel 1885

*****_Palaeorchestia parallela_ (Fritsch) 1876

*****Genus _Clarkecaris_ Messalira 1952

*****_Clarkecaris brasili_ Clarke 1920

** Indicates that these taxa are extinct and represented in the fossil record.*

Superorder SYNCARIDA Packard 1885

Diagnosis

Modified after Perrier et al. 2006, and Poore et al. 2002.

Eumalacastracan that lack a carapace; body slender; integument thin; all body segments generally subequal and free or first fused with the head; female with no oostegite development to form an incubatory marsupium; telson with uropods; antennula with three articled peduncle with two flagella; antenna with one multiarticulate flagella, with or without a endopodite modified into scaphocerite (scale-like feature); Pleonite 6 generally not fused to telson; at least some thoracic appendages biramous; thoracopod 8 in males modified as copulatory limb.

Key to the Orders

The keys are modified from Williams (1980) and Serov (2002) and are primarily designed for use with adult specimens i.e. specimens that have developed genital structures however, they will still work with juvenile specimens although some of the characters may not be as prominent, such as number of antennal segments and setae.

1.	Cephalon lack a carapace, all thoracic segments free or first fused with the head, exopodites present on walking legs; no limbs modified as gnathopods.	**Superorder Syncarida**	**2.**
2.	7 free thoracic segments (trunk with 13 body segments)	**Order Anaspidacea**.	**3.**
3.	8 free thoracic segments (trunk with 14 body segments)		**4.**
4.	Caudal furca present, extant taxa	**Order Bathynellacea**	**5.**
5.	Caudal furca absent, extinct taxa	**Order Palaeocaridacea**	

Order BATHYNELLACEA Chappuis 1915.

Diagnosis

Modified after Camacho et al. 2002

Minute syncarids (<3mm) with eight thoracic and five abdominal free segments; furca always present; uropod and pleotelson narrow and tubular and do not form a tail-fan; eyes and statocycts absent; antenna shorter than or equal to antennula thoracopods 1-7 biramous; male thoracopod 8 modified as a copulatory organ but without a petasma and reduced in female.

Order PALAEOCARIDACEA Brooks 1962

Diagnosis

Modified after Perrier et al. 2006 and Schram 1986.

Fossil syncarids with eight free thoracic segments and six abdominal segments; telson and uropods developed as a fan-like structure with strong lobate uropods; rostrum formed by anteromedial extension of cephalic plate; eyes on articulated stalks when present, antennula statocyst unknown; antenna longer than antennula; antenna endopodite (when present) modified into scaphocerite (scale-like feature); first thoracopod modified (typically reduced) as a maxilliped; all thoracic segments with five components; uropods with one endopodite and one exopodite; exopodites of uropods usually with diastema (axial cuticular thickening or a line of articulation defining the second rami segment).

Order ANASPIDACEA Calman 1904

Diagnosis

Modified from Knott & Lake 1980.

Pereonite 1 fused with head; thoracic segments 2-8 always distinct; pleon with six distinct, separate pleonites; somites generally subequal in length to cephalon; telson round, triangular, bifid or absent; uropodal with single or double segmented rami; eyes pedunculate, sessile, or absent; peduncle of antennula three segmented; statocyst present on first antennula peduncle; antenna shorter than antennula peduncle of antenna with four segments; scaphocerite scale present on 2nd segment or absent; mandible without lacinia mobilis; thoracopod 1 modified into a maxillipeds and usually larger than remaining thoracopods; thoracopods 2-8 uniform in general structure and adapted for walking; plumose, multisegmented exopodites present on basis on all but the last one or two pairs of thoracopods; 1 or 2 exposed tubular or lamelliform epipodites present on the coxae on all but the last thoracopod; pleopoda natatory (multisegmented), reduced or absent, and no appendix interna; pleopod endopodites rudimentary but usually absent, except for the first two pairs in males where they form a petasma.

Remarks

There are several changes in this definition from the previous diagnoses of Calman (1904), Sayce (1908) and Knott and Lake (1980) that pertain to the eyes, ancillary structures on the thoracopods, pleopods and telson. The diagnosis of the Palaeocaridacea by Perrier et al. 2006 states that the thoracopods segments have five components however all Syncarida have at least seven which include the coxa, basis, ischium, merus, carpus, propodus and dactylus. There may also be additional components such as the precoxa and preischium in in species such as *Anaspides*. Another change is the removal from the Anaspidacea of the mandibular processus incisivus accessorius as the structure, if it exists at all, is not a consistent feature across the order. Another feature to be added to all ordinal diagnoses is the relative length of the antenna to the antennula. The Bathynellacea and the Anaspidacea both have the antenna shorter than the antennula whereas the Palaeocaridacea have the antenna considerably longer in those fossils that have these features preserved, such as *Palaeorchestia parallela* and *Uronectes fimbriatus*.

Key to the Families of the Anaspidacea

Modified from Serov (2002).

1. Pleopods large, multisegmented and present on all abdominal segments; eyes present, reduced or absent; both uropodal rami one segmented **3.**

2. Pleopods reduced to one to two segments or absent; eyes always absent, uropodal rami uniramous or biramous **5.**

3. Telson, distally rounded or distally triangular, elongate (length > width), eyes stalked; rostrum formed from a single lobed extension of cephalic plate; uropods uniramous forming a flattened tail fan.

Family Anaspididae. (See section 2.1 for Genera and Species). **4.**

4. Telson triangular, short (length ≤ width), eyes sessile or absent, frontal margin of cephalon triangular but not lobed, uropod rami simgle segments but not forming a tail fan.

Family Koonungidae. (See Chapter 2.2 for Genera and Species). **6.**

5. Rostrum small, single or bilobed and articulated below anterior margin of cephalic capsule **7.**

6. Rostrum broad triangular extension of anterior margin of cephalic capsule but not a lobe **9.**

7. Endopodite of uropod 2-segmented

FamilyPsammaspididae. (See Chapter 2.3 for Genera and Species). **8.**

8. Endopodite of uropod 1-segmented; exopodite of uropod 1-segmented and telson reduced to a rudimentary furca.

Family Stygocarididae. (See Chapter 2.5 for Genera and Species).

9. Telson strongly bilobed and quadrangular

Family Patagonaspididae (one species) (See Chapter 2.3 for Genera and Species). **10.**

10. Telson single lobed, triangular and elongated (length ≥ width)

Family Raptornungidae n. fam (**Family A** Serov 2002) (See Chapter 2.4 for Genera and Species).

CHAPTER 2. MATERIAL AND METHODS

Field Sampling Methods

The Habitats

The Anaspidacea occur in a broad range of surface water and cryptic groundwater habitats typically in cool to cold water bodies that are permanent and linked to groundwater systems. A perennial water supply is an essential habitat criterion as the species in the order have no physiological strategies in their life cycle that can resist desiccation. The habitats include caves, hyporheic zone of (sand and gravel beds) rivers, aquifers. In Tasmania however, the Anaspididae still inhabit the surface waters of highland streams, pools and lakes. They are predominantly detrital feeders consuming sediment, alga and diatoms although many will consume animal tissue (they can also be cannibalistic) and can be collected using fish as bait. (Reik 1959., Eberhard & Spate 1995).

Surface water anaspidaceans are now limited in distribution to Tasmania and Victoria, where they are restricted to the colder waters of the higher altitudes and to small groundwater fed swamps and seeps on the west coast of Tasmania as well as the small ephemeral swamps along the southern margins and ranges in Victoria. Although Anaspides can be frequently collected from streams they are usually found within the pools rather than the flowing riffle sections. The transition from open water to an interstitial existence is reflected in the morphological reductions in appendages such as telson size and shape, the size and number of segments of the pleopods or abdominal legs and the reduction and loss of not only eye function but also eye structure as can be seen through progressive reductions in these structures from the surface dwelling Anaspididae to the intermediate Koonungidae to the to the entirely phreatic Raptornungidae, Psammaspididae and Stygocarididae. (Schminke 1974b, Coineau 2000, Coineau & Camacho 2013, Williams 1965a, b).

The Anaspidacea occupy a broad range of freshwater ecosystems which therefore require a range of collecting techniques. All anaspidaceans occur in environments that are, to varying degrees, dependant on groundwater. Where they occur in subterranean aquatic environments they belong to a community termed stygofauna. In many cases, these environments and the associated stygofauna have been shown to have high endemicity and therefore have conservation value (e.g. Smec 2006, Hose 2008, Howitz 1988, Knott & Lake 1977, and Knott & Lake 1980)

Stygofauna monitoring and assessment is a relatively new field of exploration in Australia. In the past most groundwater research has mostly been limited to physico-chemical and hydrological parameters. Very few studies have examined the ecological aspects of these diverse and complex environments, and only a few areas have been surveyed in NSW. A far more considerable amount of work has been conducted in

Western Australia over the last 20 years with the Syncarida Order Bathynellacea forming a significant component of the stygofauna community (Cho *et al*. 2005, Humphreys &Adams 1991, Humphreys 1994, Humphreys 1993, Humphries 2002, Humphreys 2001, Humphreys 1993). Most of the Groundwater Dependent Ecosystem (GDE) research to date has centred on the hyporheic (Boulton & Foster 1998, Boulton *et al*. 2004, Coleman & Hynes 1970, Growns & Williams 2006, Hancock & Boulton 2008, Hancock 2002, Hancock 2004, Schmidt *et al.* 2004, and Tomlinson *et al.* 2007) and karst environments (Clarke 2000, Clarke 2006, Crawford 1985, Doran *et al.* 2001, Dyson *et al*. 1982, Eberhard & Spate 1995, Eberhard *et al*. 1991, Eberhard, 1993, Gillieson 1996, Goede 1967, Kiernan 1988, Thurgate *et al*. 2001a, Thurgate *et al.* 2001b and Zeidler 1985). These studies have shown that GDE's support a higher than expected diversity of fauna, with many fauna having a very high degree of endemicity, and in many cases showing important phylogenetic and biogeographical relationships. This is certainly the case with the Anaspidacea.

Subterranean water occurring in aquifers constitutes approximately 97% of all the unfrozen freshwater reserves on earth and forms the largest, and possibly, most diverse freshwater ecosystems (Castany 1982, Malard *et al.* 2001). These habitats occur from the sub-arctic to the equator, from mountain range to below sea level. They are characterised by perpetual darkness and low food supplies, and relatively constant environmental variables such as low dissolved oxygen and temperature. The diverse habitats range in void size from microscopic pores through a fine sand aquifer to vast caverns in limestone cave systems. The water in these systems can be saline or fresh in a variety of water-saturated/unsaturated voids in consolidated or unconsolidated sediments (Juberthie 2000, Ward *et al*. 2000. Malard *et al.* 2001).

Detailed Methodologies

The Sampling Methodologies used for this study is set out below and divided up into three habitat types.

1) Surface Water Environments

Each site was sampled using two standardized methods outlined in the River Bioassessment Manual (Anonymous 1994) and the NSW AUSRIVAS (Australian Ri**v**er Assessment **S**ystem) sampling and processing manual (Turak *et al*. 2004).

The first method involves the rapid assessment techniques employed in NSW AUSRIVAS Sampling and Processing Manual (2000). Two main surface water habitats were sampled, 1) pool edge of rivers and lakes and 2) riffle zones of streams and rivers.

1a) Pool and lake edges are sampled with a macroinvertebrate kick net that is drawn through the water over the substrate starting from 1.5m off the edge and working in towards the bank along the edge in a rapid motion for approximately 10m. In order to sample a larger area of a river reach, smaller

samples of approximately 1-2m are taken along the length of the pool to a maximum combined length of 10 metres.

1b) Riffles are sampled with a kick net held in the riffle with the net extending downstream while the substrate directly upstream is agitated with either hands or feet The sampling strategy is the same as for edges, where, in order to sample a larger area of the riffle zone, small samples of approximately 1-2m are taken along the length of the riffle to a maximum combined length of 10 metres.

2) The second method involves targeted opportunistic sampling from a variety of habitats and substrates, when available. Habitats sampled may include:

- logs and wood from within pools, pool edges and riffles;
- large rocks and boulders from within pools and riffles;
- kick net sweeps through macrophyte beds and;
- kick net sweeps under bank overhangs;
- small hand net sampling of small pools and seeps.

2) Bore/Peizometer Sampling - The Phreatic/Hypogean Zone

The phreatic zone is the subsurface area within an aquifer where voids in the rock are completely filled with water. This environment is occupied by phreatobites – i.e. stygofauna that are restricted to the deep groundwater substrata of alluvial, fractured rock and karst aquifers (phreatic waters) and in areas of shallow groundwater where groundwater fauna form a significant component of the 'Pholeteros community that occupy the burrows of land crayfish (Creaser 1931, Lake 1977). Access to this environment is through water bores or by pumping out crayfish burrows. The stygofauna community was sampled using three standardised methods.

a) The first technique is the standard technique that has been used successfully overseas and in Australia (Cvetkov (1968), Bou 1974, Humphreys 1994, Schmidt *et al.*2004). This involved using a weighted long haul or plankton net with a 100-300 μm mesh with a diameter that is slightly smaller than the diameter of the bore or piezometers. Sampling consisted of dropping the net down to the bottom of the bore and taking at least three consecutive hauls from the entire water column at each bore. Upon removal from the bore the net is washed of sediment and animals and the contents of the sampling jar (the weighted container at the bottom of the net) are decanted through a 150 μm mesh sieve. The contents of the sieve are then transferred to a labelled sample jar and preserved with 100% ethanol.

b) The second method is the use of a water bailer. A bailer is typically used by hydrogeologists to taken water samples from bores for water quality/water chemistry analysis. The bailer used for this study is a 1

meter long by 40mm wide stainless steel tube with a running ball valve at the bottom. The size of the bailer, and net for that matter, is determined by the diameter of the bore. The advantage of using a bailer is twofold. The main reason for using a bailer is that it is able to sample the bottom sediment of a bore that cannot be sampled by a haul net and therefore enables the collection of cryptic invertebrates that do not inhabit the water column or sides of the bore. The second advantage is that in shallow bores down to 5 meters in sediments with low transmissivity or porosity a bailer is able to empty the entire contents of a bore and thereby confidently collect all animals within the bore.

The contents of the bailer are emptied into a cleaned bucket from which the water is then decanted through a 150 μm mesh sieve. The contents of the sieve are transferred to a labelled sample jar and preserved as above. Following sampling and preservation of the sample and prior to the next sampling at a new location all equipment including the bailer, net and sieves must be rinsed clean with clean water via a spray bottle to remove any sediment and animals that may have remained attached to the sampling devices. This is to reduce the possibility of cross contamination of organisms (stygofauna or bacteria) or pollutants from one aquifer or bore to another.

c) The third method is specifically for sampling the pholeteros of land crayfish burrows. This involved carefully digging out the burrow to the standing water level and pumping out the water using either a small hand pump and filtering the water through a 150 μm mesh sieve. The contents of the sieve are then transferred to a labelled sample jar and preserved with 100% ethanol.

3) River Bed Sampling - The Hyporheic Zone (Figure 2.1-3)

The Hyporheic Zone is the ecotonal zone below and within the porous sand and gravel substrate of a riverbed. The fauna that inhabits this ecotone is thus termed the hyporheos. The hyporheos represents a diverse range of organisms from surface macroinvertebrates to groundwater invertebrates depending on the depth and origin of the water parameters i.e. whether the water is groundwater or surface water dominated.

The hyporheic zone is a highly variable and fluid ecotone that is controlled by the river flow rates, groundwater baseflow in gaining streams, sediment type, sediment porosity and water chemistry/quality. Gaining streams are those where the surrounding groundwater is higher than the stream bed, therefore the water flows from the groundwater into the stream. This high variability in physical parameters has been shown in previous studies (Boulton et al. 2003, Boulton et al. 2004, Danielopol 1989, Growns & Williams 2006, Ward 2000) to be reflected in the distribution of elements of the hyporheic fauna. The Syncarida have been found to occur in streams characterised as being groundwater fed, have a course sand/gravel composition and are generally permanent. They have also been found to occur most commonly and in higher numbers from the bottom of riffles zones (Boulton 2003, 2004). This area has been shown to

contain the highest concentration of groundwater fauna (Boulton 2003, 2004) as it represents the upwelling zone of a river bed as opposed to the top of a riffle which is the downwelling zone. Anaspidaceans have also been collected from the sides of streams in gravel beds (Schminke 1974a).

Figure 2.1. Demonstration of the Bou Rouch Pump by Dawit Berhane used to sample Psammaspididae in the hyporheic zone in Maules Creek, NW NSW.

Figure 2.2. The author sampling a peizometer with a bailer and phreatobiology net on the Peel River, near Tamworth, northern NSW.

Figure 2.3. Bou Rouch pump set up for sampling the hyporheic zone of streams.

Each hyporheic sample is taken using the Bou-Rouch Pump method (Bou and Rouch 1967, Bou 1979, Bou 1974) as demonstrated in Figures 2.1 and 2.2, which is similar to the method used by Boulton *et al.* 2003, and Boulton *et al.* 2004). The principle of the method is to drive a pipe or spear point into the substrate and to extract water and animals via an attached pump. This method creates a pressure and flow alteration within the substrate which disturbs the sediment and maintains an interstitial flow around the pipe that is sufficient to dislodge subsurface invertebrates (Malard *et al*, 2001, Bou and Rouch 1967). A hollow plastic pipe with a 16 mm internal diameter was hammered between 0.5-1.5 m into the riffle substratum. After attaching a hand pump up to 30 litres of water is pumped from the riffle into a bucket and the sample was then filtered through a 150µm mesh. All animals and material collected was then preserved in 100% ethanol. Up to four samples were taken from each riffle site.

4) Spring Sampling - The Groundwater Discharge Zone

Springs represent a window into the groundwater environments and are the ecotones between groundwater and surface water ecosystems. They can contain mixed community assemblages of organisms from the groundwater (phreatic) zone, the epigean (surface water) zone and fauna that is specifically adapted to springs (crenobiont taxa) (Malard *et al.* 2001). There are many types of springs from the commonly perceived point source discharge zone with free flowing water out of a hole to extensive slow seepage zones that can be gravity fed springs to artesian pressure fed springs. Flow can be permanent or intermittent. (Malard *et al.* 2001). Springs may be either fed by groundwater from unconsolidated sediments or through fractures or faults in bedrock or dissolution voids in karstic systems. In order to adequately sample springs it is necessary to use a combination of techniques due to the strong within and between site community heterogeneity. The techniques used include:

Sampling the benthic layer around the spring using a) a standard aquatic macroinvertebrate net including the bed sediment, and b) hand collecting from any allochthonous material such as woody debris and collecting and washing leaf litter and aquatic vegetation such as mosses.
The use of drift nets such as a surber sampler at or downstream of the spring outlet is useful in collection drifting fauna. These nets are set over a period of time from an hour to overnight.

Measurement of Physico-Chemical Variables

Physical and chemical variables were measured at each collection site insitu where possible. The variables included temperature, dissolved oxygen, conductivity, ph and depth to standing water (in metres) within bores. The units used for each of the above mentioned water quality variables are as follows.

Parameter	**Units**
Temperature	**°C**
Dissolved Oxygen	**mg/l**
Conductivity	**µS/cm**
PH	**ph units**
Depth to water	**Metres**

Table 2.2. Physico-chemical environmental variables

Laboratory Methods - Dissection and Description

Preservation

All samples are preserved and labelled in the field with 100% ethanol and returned to the laboratory where each sample is sorted from the collected sediment under a stereomicroscope and stored in 100% alcohol. The specimens were preserved in 100% ethanol to enable the specimens to be included in later DNA analysis studies.

Methods of Dissection, measurements and illustrations

All specimens are preserved in 100% ethanol and were prepared and mounted in glycerin. For sorting, dissection and preparation of specimens, a Leica MZ12 Microscope was used. Whole specimens were drawn using a Leica MZ12 stereomicroscope fitted with a camera lucida and a Leica eyepiece graticule for measurements. Appendages and mouthparts were then dissected and mounted on slides in glycerol and drawn using a camera lucida fitted to a Leica DMLB compound microscope with differential interference contrast equipment and oil immersion. Once the drawing was complete the image was scanned and corrected using Adobe Photoshop 4. Photographs of the Anaspididae and other whole animal were taken using a Leica MZ12 mounted with a Leica digital camera.

Anaspidacea Site Records

The Anaspidacea records data used within this thesis and presented with each of the descriptions was compiled from a range of sources including published literature, museum records, government agency records and personal collection records. The data presented is that which was provided with each sample, survey record or each species description and therefore may vary widely in accuracy and confidence from highly detailed site descriptions and coordinates to very vague descriptions without details. As each site could not be visited to confirm the accuracy of the record no confidence rankings can be given. Some records have data missing such as collection dates or coordinates. This is a particular problem with many of the early records and are, therefore of limited distributional value. The earlier records do however, have historical value, therefore all records are included for completeness. These listings include the following categories:

Family and Species Name – This is the name given with the original data.

Locality – A brief description of the site from which the specimens were collected.

Altitude: This data is based on either original records or estimated using maps or Google Earth from the geographic coordinates given. In some records this could not be determined.

Latitude and Longitude – Spatial coordinates have been standardized in decimal degrees. As the records spanned large time periods, cultures, individuals, agencies, states and countries, the original locality coordinates were recorded using a number of different methods which therefore, required them to be converted to a standardized format.

Collection Date – The dates given are from the original sources. For some records there are no precise dates available.

Data Source – This is the source where the locality data is referenced in the literature or from individuals (pers com.) and/or location where the specimens or collections are housed.

These include:

Australian Museum
Boulton, A & Cord, J. (pers com.)
Boulton, A & Lisle, P. (pers com.)
Depart of Primary Industries & Water, Tasmania.
Eberhard, S.M. (pers com.)
EPA Victoria
Flynn, 1918 (See references)
Fulton. W., Davies, P. (pers com.)
Fulton. W., Hall. J., Ponder. W. (pers com.)
Grosso & Peralto 1997. (See references)
Grosso & Peralto 1997. (See references)

Grosso & Peralto 2002. (See references)
Hancock. P. (pers com.)
Hickman 1937. (See references)
Hose, G. (pers com.)
Howitz, P 1988. (See references)
Inland Fishers Commission, Hobart, Tas
Jarman, S. N. (pers com.)
Jarman, S. N., Elliott, N.G. (2000). (See references)
Knott & Lake 1980. (See references)
Korbel, K. (pers com.)
Lake & Coleman, 1977. (See references)
Morimoto 1977. (See references)
Murray Darling Freshwater Research Centre, Albury, John Hawking
Museum of Victoria
Nicholls 1931. (See references)
Nicholls 1947. (See references)
Noodt 1963b. (See references)
O'Brien 1990. (See references)
Queen Victoria Museum
Sayce 1907 (See references)
Sayce 1908. (See references)
Schminke 1980. (See references)
Serov 1988. (See references)
Serov. P. (pers com.)
Sloane. T. (pers com.)
South Australian Museum
Swain, R., Reid, C, 1983. (See references)
Swain, Wilson, Hickman & Ong 1970. (See references)
Swain. R, Wilson & Ong 1971. (See references)
Tasmanian Monitoring River Health
Tasmanian Museum & Art Gallery
University of Tasmania (Dr. R. Swain)
Vic AUSRIVAS EPA
Watts, Hancock, Leys 2007. (See references)
Whinam, J., Eberhard, S., Kirkpatrick, J., & Moscol, T 1989. (See references)

Williams, W.D. 1965. (See references)
World Heritage Area Reports 1987-89. (See references: Fulton, W. and Howitz, P. (1987); Fulton, W. (Ed) (1988); Chilcott, S. (1989);)
Zeidler 1985. (See references)

Table 2.3. List of data sources.

Distribution maps

The distribution maps presented in this thesis were created using: 1) Manifold 8 Professional GIS program produced by Manifold Software Limited, and 2) Google Earth.

Abbreviations

Institutional Abbreviations

Prefixes of registration or catalogue numbers for the Institutions referred to in the text, tables and figures.

AM - Museum d'Histoire Naturelle, Autun, France

B - Museum d'Histoire Naturelle, Paris, France

BS - Bayerisches Staatssamlungen für Paläontologie und historisches Geologie, Munich, West Germany

CGH - Národni Museum, Prague, Czechoslovakia

FML - Museo Civico di Storia Naturale di Verona, Italia and Fundaciòn Miguel Lillo, Argentina.

G - Tasmanian Museum and Art Gallery, Hobart, Australia

GSL - Institute of Geological Sciences, Leeds, England

I, In - British Museum (Natural History), London, England

IFC - Inland & Fisheries Commission, Hobart, Tasmania, Australia

ISGS - Illinois State Geological Survey, Urbana, Illinois, USA

Jk - Museum für Naturkunde (Paleontologisches Museum Catalog), Berlin, East Germany

M, Me - Národni Museum, Prague, Czechoslovakia

NB - Rijks Geologische Dienst, Heerlen, The Netherlands

NMV - Museum of Victoria, Melbourne, Australia

NYSM - New York State Museum, Albany, New York, USA

P or F - Australian Museum, Sydney, Australia

PE - Field Museum of Natural History, Chicago, Illinois, USA

PMB - Museum für Naturkunde (Paleontologisches Museum Catalog), Berlin, East Germany

QVM - Queen Victoria Museum and Art Gallery, Launceston, Australia

SAM - South Australian Museum, Adelaide, Australia

SDSNH - San Diego Natural History Museum, San Diego, California, USA

TM - Tasmanian Monitoring River Health, Tasmania, Australia

US - University of Sydney, Paleontology Collection, Sydney, Australia

USNM - National Museum of Natural History, Smithsonian Institute, Washington D.C., USA
UT - University of Tasmania (Dr. R. Swain), Hobart, Tasmania, Australia
VEPA - Victorian AUSRIVAS EPA, Melbourne, Victoria, Australia
X - University of Illinois, Paleontology Collection, Urbana, Illinois, USA
YPM - Yale Peabody Museum of Natural History, New Haven, Connecticut, USA.

Morphological Abbreviations

The abbreviations used in the figures and tables are:
Cephalon - C; Rostrum - R; Antennula - A.I; Antenna - A.II; Labrum - Labr; Mandible – Md; Paragnath - Pg; Maxilliped (Thoracopod 1) – Mxp; Maxillule - Mx1; Maxilla - Mx2; Thoracopods 1, 2 etc. - Th. 1, 2, etc..; Female seminal receptaculum – SR; Pleopods 1, 2 etc. - Pl 1, 2, etc.; Telson - T; Uropod 1, 2, etc. – U1, 2, etc.

Morphological Terminology

Due to the variability in terminology used for the Syncarida over time, one of the aims of this study was to standardise the terminology used in order to have a consistent approach across the families. The terminology used in this study for setae and spines is that as described by Watling (1989) although Peralto (2010) has updated the setal terminology, particularly in relation to the Stygocarididae and Patagonaspididae. The descriptions and diagnoses follow a standardised order in which the animal is described. The body size, form and structure of the major segments are described first from anterior to posterior, i.e. cephalon, thorax, abdomen, followed by the appendages from anterior to posterior i.e. mouthparts, thoracopods, pleopods, uropods.

Body segments are described as pereonites 1-7 and pleonites 1-6 or generic somites while the lateral extensions are termed pereomeres and pleomeres or generic epimeres or epimera. The thoracopoda, pleopoda are numbered 2-8 and 1-5 respectively rather than using Roman Numerals for the thoracopoda and numbers for the pleopoda as is often the case in such groups as the Pericarida. Component pieces of articulated appendages such as the antennal flagellum are composed of segments and not articles as is often used. The body is divided into anterior (front) and posterior (back), and medial (middle) and lateral (side) elements, i.e. Inner and outer margins are described as medial or lateral margins. In addition, a segment will have a distal (tip) and proximal (basal) end. Cephalon antennae are termed antenna for the lateral antennae and the antennula for the medial antenna. Mouthparts are described as maxillula (maxilla 1/Mx1) and maxilla (maxilla2/Mx2). I prefer to use the terminology for the first set of legs as maxilliped rather than thoracopod 1 due to the associated function with the mouthparts. The thoracic legs are described as thoracopods rather than pereopods which is the term typically used to describe these limbs in the Pericarida. The sixth abdominal segment has a pair of modified pleopods termed uropoda that consists of a

basal protopod with an endopodite and exopodite each with 1-2 segments or rami. The posterior body segment attached the posterior margin of pleonite 6 is the telson.

The telson region of the Stygocarididae differs from the other families in that the telson is significantly reduced or absent. New terminology is presented here to describe the telson/anal operculum (telson or telsonic plate) region. The telsonic plate has a variably shaped, sinuous distal margin, but does not cover the lateral and distal margin of the lateral lobes of the anus. The 'anal lobes' is a new term first used here to describe the broad lobes positioned under the telsonic plate with each subdivided into an anal 'lobula globosa', which are positioned on each side of the anal opening and the 'lobular rudimenta', which are raised mounds that bear lateral setae.

Niche Adaptation Terminology Used in This study

The aquatic fauna presented in this study are classified by following the system of Gibert, *et al.*1994 that classifies an animal based on its morphological and physiological adaptations to existing in groundwater environments. All aquatic fauna, with a specific relevance to invertebrate fauna, can be classified by the degree to which they are dependent on groundwater. The degrees of adaptations range from being completely dependent on groundwater (stygobites) and consist predominantly of crustaceans, to those that are not adapted to survive in groundwater environment (stygoxenes). The distinction is often ambiguous because it is difficult to know the degree of surface/groundwater mixing in an aquifer (Boulton, *et al.* 2004).However, classifications based on affiliation to groundwater can be useful (Marmonier, *et al.* 1993) when assessing the conservation status of species and their vulnerability to potential impacts. In this thesis we adopt the following definitions:

Stygoxenes - organisms that have no affinities with groundwater systems but regularly occur by accident in caves and alluvial sediments. Some planktonic groups (e.g. Calanoida Copepoda) and a variety of benthic crustacean and insect species (e.g. Simullid larvae, Caenidae Mayflies) may passively infiltrate alluvial sediments (Gibert *et al.* 1994).

Stygophiles – organisms that have greater affinities with the groundwater environment than stygoxenes because they appear to actively exploit resources in the groundwater system and/or actively seek protection from unfavourable surface water conditions. The stygophile groups can be separated further into 'occasional' and 'permanent' hyporheos (Williams & Hynes, 1974), and the organisms termed as 'amphibites' by Ward, *et al*. (1994) can be recognised.

Occasional or Temporary Hyporheos

The occasional or temporary hyporheos include individuals that could either spend their lives in the surface environment or spend a part of their lives in the sub-surface environment of the hyporheic zone of rivers and streams (e.g. Ceratopogonidae larvae). The hyporheic zones is the environment within the unconsolidated sediments of water courses and can include sand and gravel beds, course organic sediments (Gibert *et al.* 1994).

Permanent Hyporheos

The permanent hyporheos is present in the hyporheic zone during all life stages in either groundwater or benthic habitats (Gibert *et al*. 1994) and possess specialist adaptations for living in this environment (Gibert et al. 1994).

Stygobites - obligate subterranean species, restricted to subterranean environments and typically possessing specialised character traits related to a subterranean existence either within the subterranean terrestrial environments of caves/karst and Fracture rock terrains (troglomorphisms) or subterranean aquatic environments (Gibert *et al.* 1994).These morphological traits or adaptations can include: reduced or absent eyes and pigmentation; enhanced non-optic sensory structures. Within the stygobite group are 'ubiquitous' forms (found in karst and alluvial aquifers and also occasionally at or close to the stream surface), as well as forms restricted to deep alluvial aquifers ('phreatobite').

Phreatobites - specialist stygobites that are restricted to the deep groundwater substrata of alluvial aquifers (phreatic waters). All species within this classification have specialised morphological and physiological adaptations (Gibert *et al*. 1994).

Stygofauna - an all-encompassing term for animals that occur in subsurface waters (Ward, *et al*. 2000).