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**Molecular and morphological evidence for short range endemism
in the *Kinnecaris solitaria* complex
(Copepoda: Parastenocarididae),
with descriptions of seven new species**

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Abstract

Recent investigation of one of the larger calcretes in the uppermost reaches of the Carey palaeochannel in the Yilgarn region of Western Australia revealed an unprecedented diversity of copepod crustaceans. Twenty-two different species and subspecies, from six copepod families, represent 70% of the previously recorded copepod α -diversity in the whole region, although the area investigated is less than 3% of its surface. The aims of this study were to explore the diversity of the harpacticoid genus *Kinnecaris* Jakobi, 1972 using both molecular and morphological methods, establish precise species boundaries, find their accurate area of distribution, reconstruct phylogenetic relationships, and explore colonisation pathways. To achieve this we sampled very intensively in the area, as well as in two neighbouring palaeochannels, analysing more than 700 samples from 230 different localities, half of which contained copepods. Seven species are described here as new, five of them from the Yeelirrie palaeochannel (*K. esbe* **sp. nov.**, *K. lined* **sp. nov.**, *K. linel* **sp. nov.**, *K. linesae* **sp. nov.**, and *K. uranusi* **sp. nov.**) and one each from two neighbouring palaeochannels (*K. barrambie* **sp. nov.** and *K. lakewayi* **sp. nov.**). *Parastenocaris jane* Karanovic, 2006 from the Pilbara region, along with a newly described third Australian parastenocaridid genus from the Yilgarn, were used as outgroups in our molecular analysis. The COI fragment was successfully PCR-amplified from 12 parastenocaridid specimens using a nested combination of primers. All analyses supported the presence of at least seven genetically divergent lineages, most supported with very high bootstrap values. Three genera formed three separate clades, and the average pairwise distances between *Kinnecaris* morpho-taxa were found to be very high (8.2-16.8 %), while the highest divergences within morpho-taxa were 0.3%. Some conflict between molecular phylogenies and morphological data was observed when it came to recognizing different groups of species. While morphology indicates that *K. esbe*, *K. linel*, and *K. uranusi* represent a group of very closely related species, supported by a number of synapomorphies, molecular analyses suggest that *K. linel* and *K. uranusi* are only remotely related. We argue

in favor of morphological data, until more markers can be studied to try to resolve these differences. In Yeelirrie, morphological evidence would suggest a downstream colonisation history in the genus *Kinnecaris*, where the most plesiomorphic form (*K. linesae*) lives in the uppermost reaches of the palaeochannel, and the trend in the caudal rami elongation and denser somite ornamentation is obvious downstream the palaeochannel (*K. uranusi*, *K. linel*, and then *K. esbe*), with the only exception being *K. lined*, which probably represents an independent colonisation event. Parastenocarids are copepods of freshwater origin, and we argue that they can probably disperse downstream during periods of increased rainfall, evolving into separate species in isolated calcrete pockets during periods of increased aridity. Although some of the questions remained unanswered in this study, detailed morphological and molecular observations indicate that we are not dealing with one widely distributed and variable species in the Yilgarn region, but rather with a complex of short range endemics. Areas of distribution for different species range from 30 km to less than 5 km in diameter. Very strong seasonal dynamics in this subterranean community was observed, and this is a novel concept for these ecosystems globally. A key to nine Australian species of *Kinnecaris* is also included.

Key words: Western Australia, Harpacticoida, stygofauna, taxonomy, systematics, barcoding

Introduction

Family overview. The family Parastenocarididae Chappuis, 1940 is a monophyletic group within Harpacticoida, being easily distinguished by the sexual dimorphism in the third pair of swimming legs (Corgosinho et al. 2007). Modification of these legs in males into a grasping organ, that allows them to hold females during copulation (Glatzel 1996), is one of the most important synapomorphies of the group (Martinez Arbizu & Moura 1994), but many other morphological characters make it very easy to instantly recognize its members (Karanovic & Cooper in press). However, a great number of morphological characters are conservative within this family, making generic division a real and long lasting problem (Reid 1995; Galassi & De Laurentiis 2004; Karanovic 2005; Schminke 2010), and the family stayed monogeneric for a long time despite a steady accumulation of new species.

Chappuis (1937) divided its only genus *Parastenocaris* Kessler, 1913 into four groups, which he numbered rather than named, each containing two species. Kunz (1938) added another group. Lang (1948) subdivided the family into eight species-groups for 31 of the 40 species known at that time (nine species were either known only as females or were insufficiently described), accepting the group proposed by Kunz (1938), but rearranging three of those proposed by Chappuis (1937) and naming them after the most characteristic species. For diagnosing all these groups all three authors mostly used characters of the male fourth leg endopod. Despite being chiefly based on a single character, Lang's system was widely accepted and was coping rather well with a subsequent steady influx of newly described species from around the world, culminating in the decade between 1963 and 1972 when 75 new species were added (Schminke 2010). Five new species groups were added subsequently by Noodt (1962, 1963, 1972), mostly for the newly discovered and very diverse South American fauna, but it became apparent that this increasingly more complex system of species groups was not a reflection of true phylogenetic relationships, which were not taken into account in the description of many of the new taxa.

Jakobi (1969) described one of the Noodt's groups as a new genus, and it was Jakobi (1972) who made the first effort to revise the family by splitting it into 26 different genera (although only assigning to them 98 out of the 155 known species). This system was strongly criticised by Schminke (1976), and was ignored for a long time by most subsequent taxonomists working on this group, all of them accepting only two of Jakobi's genera (see Por & Hadel 1986; Dussart & Defaye 1990; Reid 1995; Karanovic & Bobic 1998; Ranga Reddy 2001; Galassi & De Laurentiis 2004; Boxshall & Halsey 2004; Karanovic 2005, 2006; Cottarelli et al. 2006, 2007, 2008; Wells 2007; Ranga Reddy & Defaye 2007, 2009; Huys 2009). Jakobi (1972), for example, divided the *brevipes*-group of Lang (1948) into five different genera, which was shown by Reid (1995) to be a group of very closely related species. She even demonstrated that the type species of one new genus proposed by Jakobi is in fact a junior subjective synonym of the type species of *Parastenocaris*. Nevertheless, new genera were proposed for some unusual new members from South America (Dussart 1979; Reid 1994), Europe (Galassi & De Laurentiis 2004), Africa (Schminke 2009), Asia (Cottarelli et al. 2010), and Australia (Karanovic & Cooper in press), and two more groups of species were proposed by Berera & Cottarelli (2003) and Galassi & De Laurentiis (2004). Recently, some researchers (Corgosinho & Martinez Arbizu 2005; Schminke 2008; Corgosinho et al. 2010) started to revalidate and redefine some genera originally proposed by Jakobi (1972), as most of them remained valid and available names under the rules of the ICZN (1999), while at the same time synonymising some others.