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First cytogenetic study of a member of the harvestman family Pettalidae (Opiliones: Cyphophthalmi)

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Abstract We present the karyotype of the Australian harvestman *Austropurcellia arcticosa* (Cantrell, 1980) (Arachnida: Opiliones: Cyphophthalmi: Pettalidae), which represents the first cytogenetic analysis of Opiliones from the Australasian ecozone. The diploid complement comprised 30 chromosomes, as reported earlier for two other species of Cyphophthalmi in the family Sironidae, but with a predominance of biarmed chromosomes in *A. arcticosa*. Morphologically differentiated sex chromosomes were not observed. The course of meiosis is described in the suborder Cyphophthalmi for the first time.

Key words Australia, *Austropurcellia*, chromosome, harvestman, karyotype, meiosis.

INTRODUCTION

Opiliones is the third largest order of the arthropod class Arachnida with c. 6500 described species (Pinto-da-Rocha *et al*. 2007; Kury 2012) divided into four suborders: Cyphophthalmi, Eupnoi, Dyspnoi and Laniatores (e.g. Pinto-da-Rocha *et al*. 2007). Despite their high species diversity and widespread distribution (Cyphophthalmi are found in all continents except Antarctica, Eupnoi in both hemispheres, Dyspnoi in the Northern Hemisphere and Laniatores in all continents except Antarctica but with a peak of diversity in the Southern continents, e.g. Pinto-da-Rocha *et al*. 2007), only 1.5% of harvestman species have been surveyed cytogenetically (Tsurusaki 2007; Schneider *et al*. 2008; Rodríguez-Gil & Mola 2010). Moreover, most of our cytogenetic knowledge is derived from Eupnoi (c. 70% of all analysed species), and the majority of the information has been obtained from the Holarctic region with the exception of nine Laniatores and one Eupnoi from the Neotropics, and one Eupnoi from India (Oliveira *et al*. 2006; Tsurusaki 2007; Schneider *et al*. 2008; Rodríguez-Gil & Mola 2010).

Although harvestmen exhibit a considerable range of diploid number from 10 to 109 (Tsurusaki & Cokendolpher 1990; Oliveira *et al*. 2006), morphologically differentiated sex chromosomes have only been found in Eupnoi and Dyspnoi (Tsurusaki 1989, 2007). Except for the morphologically well-differentiated XY sex chromosomes in several species, it is suggested that ZW sex chromosomes occur in *Mitopus morio* (Fabricius 1779) and *Odiellus aspersus* (Karsch 1881) (Tsurusaki & Cokendolpher 1990). To help understand karyotypic evolution in the order Opiliones, it is essential to document data in members of the suborder Cyphophthalmi, the sister group to all other Opiliones (Giribet *et al*. 2010). However, within this suborder, only fragmentary information is available for two European species, *Siro rubens* (Latreille 1804) (Juberthie 1956) and *Parasiro coiffaiti* (Juberthie 1956) (Tsurusaki 2007). Cyphophthalmi is divided into six families with an ancient disjunct distribution (e.g. Giribet 2000; Boyer *et al*. 2007; Giribet *et al*. 2010) that correspond to three phylogenetic clades: Scopulophthalmi, Sternophthalmi and Boreophthalmi (Giribet *et al*. 2012).

The previously karyotyped species belong to the Laurasian family Sironidae, both with $2n = 30$, which is a relatively derived cyphophthalmid family (Giribet *et al*. 2012). But, species belonging to different phylogenetic lineages and distinct biogeographical regions can reveal different trends in chromosome evolution. That is the reason why we report the karyotypic contribution of a third cyphophthalmid species belonging to the southern hemisphere family Pettalidae (Scopulophthalmi), which constitutes the sister group to all other cyphophthalmids (Giribet *et al*. 2012).

MATERIALS AND METHODS

Male specimens of *Austropurcellia arcticosa* (Cantrell 1980) were sifted from rainforest leaf litter between Cape Tribulation

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and Thornton beach in proximity to the Cape Tribulation road in north-eastern Queensland (16°08′S 145°26′E). The specimens are deposited in the Western Australian Museum in Perth, Australia (WAM T104106, T104108, T104110).

The chromosome spreading technique described by Traut (1976) and modified according to Šťáhlavský and Král (2004) was used. The dissected gonads were hypotonised in 0.075 M KCl for 15 min and fixed in methanol : glacial acetic acid (3:1) for 20 min. The fixed gonads were suspended in a drop of 60% acetic acid on a microscope slide, and a drop of suspension was moved to the surface of the slide with a tungsten needle on a warm histological plate at 45°C. The chromosome preparations were stained with 5% Giemsa solution in Sörensen phosphate buffer (4.75 g $Na_2HPO_4.12H_2O$ and 4.54 g KH_2PO_4 in 1000 ml H_2O, pH = 6.8) for 35 min.

The chromosomes were observed by Olympus AX70 microscope and photographed with an Olympus DP72 camera. The measurements of the chromosomes were taken in all three males (number of measured cells were 20, 5 and 5) from photographs using the software ImageJ 1.45 s with the plugin Levan. Chromosome morphology classification follows Levan *et al*. (1964), and the relative chromosome length was calculated as a percentage of the haploid set according to previous cytogenetic studies on harvestmen (e.g. Tsurusaki & Cokendolpher 1990). Two metaphase II plates in contact were specified as sister cells and due to conspicuous centromeres were used to obtain karyotype data (Fig. 1a) because the positions of the centromeres were not well-defined in the mitotic metaphase (Fig. 1b).

RESULTS

The male diploid complement of *Austropurcellia arcticosa* comprised 30 chromosomes. The karyotype based on sister plates of metaphase II (Fig. 1a) contained three pairs of metacentric (No. 3, 9, 15), eight pairs of submetacentric (No. 1, 4, 5, 7, 8, 10, 11, 14), three pairs of subtelocentric (No. 2, 6, 12) and one pair of acrocentric (No. 13) autosomes. Autosomes gradually decreased in size from 9.5% to 5.7% of the haploid set to the 12th chromosome pair. In contrast with the small differences in size between the majority of chromosomes, the last three pairs of chromosomes were distinctly shorter than the others. These chromosomes form 4.2%, 2.5% and 1.8% of the haploid set only.

Morphologically distinct sex chromosomes or heteromorphic bivalents were not detected in males of this species. During interphase, chromosomes are isopycnotic (Fig. 1c), and during leptotene, they start to condense and begin to be visible threads of individual chromosomes with superspiralised knobs showing positive heteropycnosis (Fig. 1d). These knobs persist during zygotene (Fig. 1e) and pachytene, (Fig. 1f) but starting from diplotene (Fig. 1g), the structure of the whole chromo-

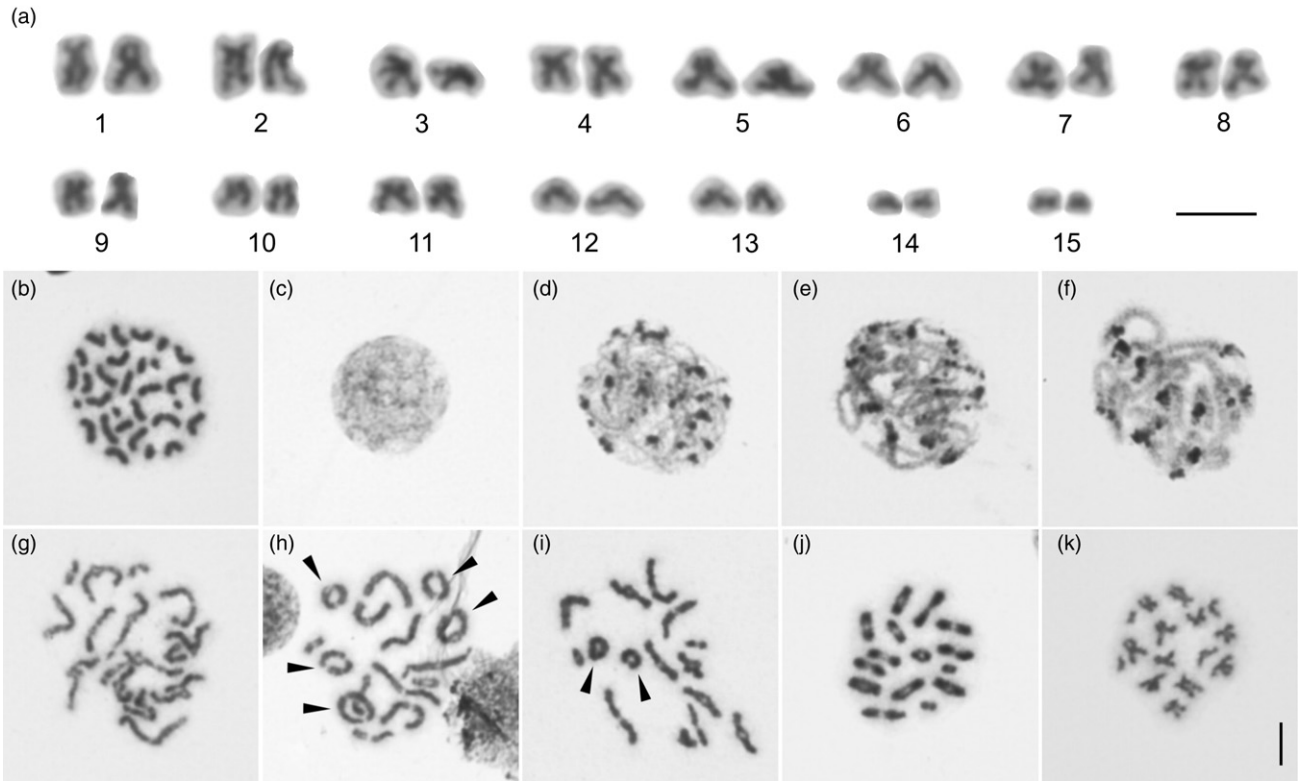

Fig. 1. Male chromosome plates of *Austropurcellia arcticosa*: (a) male karyotype (based on sister metaphase II plates); (b) mitotic spermatogonial metaphase; (c) interphase; (d) leptotene; (e) zygotene; (f) pachytene; (g) diplotene; (h) diplotene, arrows show five bivalents with two chiasmata; (i) diakinesis, arrows show two bivalents with two chiasmata; (j) metaphase I; (k) metaphase II. Scale bar = 5 μm.

somes is isopycnotic without distinct knobs. Half (50%) of the observed diplotene ($n = 26$) showed bivalent with one terminal or subterminal chiasma. However, two terminal chiasmata per bivalent that form a ring configuration were detected in one (four cases), two (eight cases) or even five (one case) bivalents per one cell (Fig. 1i,h). During metaphase I (Fig. 1j) and metaphase II (Fig. 1k), the chromosomes are not heteropycnotic.

DISCUSSION

The most abundant arachnid orders express considerable karyotype variability: mites n = 2–2n = 34 (Oliver 1977; Weeks *et al*. 2001); spiders: 2n = 7–114 (Suzuki 1954; Král *et al*. 2011); scorpions: 2n = 5–175 (Shanahan 1989; Schneider *et al*. 2009); pseudoscorpions: 2n = 7–143 (Šťáhlavský *et al*. 2006; Šťáhlavský *et al*. in press). The individual phylogenetic lineages of these orders display a trend of karyotype evolution accompanied with specific chromosomal rearrangements and with independent differentiation of the sex chromosomes (e.g. Troiano 1990; Král *et al*. 2006, 2011).

Harvestmen also exhibit a considerable range of diploid number from 10 to 109 (Tsurusaki & Cokendolpher 1990; Oliveira *et al*. 2006). However, a lower chromosome number seems to be characteristic of the basal lineages: Cyphophthalmi (2n = 30), Eupnoi (2n = 10–52, median = 22) and Dyspnoi (2n = 10–28, median = 16) (see Tsurusaki 2007). Within harvestmen only, the most derived Laniatores exhibit high numbers from 2n = 40 (Suzuki 1966) to 2n = 109 (Oliveira *et al*. 2006) that represents a derived state probably produced from polyploidy or centric fissions (Schneider *et al*. 2009).

The hypothesis of an ancestral karyotype represented by lower chromosome numbers (Schneider *et al*. 2009) may be slightly modified by our analysis. If Cyphophthalmi is the basal-most lineage of harvestmen (Fig. 2), as found in the majority of recent analysis (e.g. Giribet *et al*. 2010), the ancestral karyotype of this order may be composed of c. 30 mainly biarmed chromosomes that gradually decrease in size. In Eupnoi and Phalangioidea (Eupnoi), the chromosome number was probably independently reduced to 2n = 10 (see Tsurusaki 2007), whereas in Gonyleptoidea (Laniatores), the chromosome number conspicuously increased up to 109 (Oliveira *et al*. 2006).

Austropurcellia arcticosa has the same diploid number (2n = 30) as the two other analysed cyphophthalmids (Juberthie 1956; Tsurusaki 2007), both members of the more derived family Sironidae. However, notable differences were detected, i.e. the presence of only acrocentric chromosomes in *Siro rubens* (Juberthie 1956) and biarmed chromosomes in *A. arcticosa*. Biarmed chromosomes predominate in karyotypes of most harvestmen (e.g. Tsurusaki & Cokendolpher 1990; Tsurusaki 2007), and so the acrocentric chromosomes in *S. rubens* may be derived. However more cyphophthalmid species must be analysed to further test this hypothesis, and they could constitute a novel set of characters to resolve important phylogenetic questions such as the possible paraphyly of Sironidae (Giribet *et al*. 2012) or the position of autapomorphic species such as *Shearogovea mexasca* (Shear 1977) (Giribet 2011).

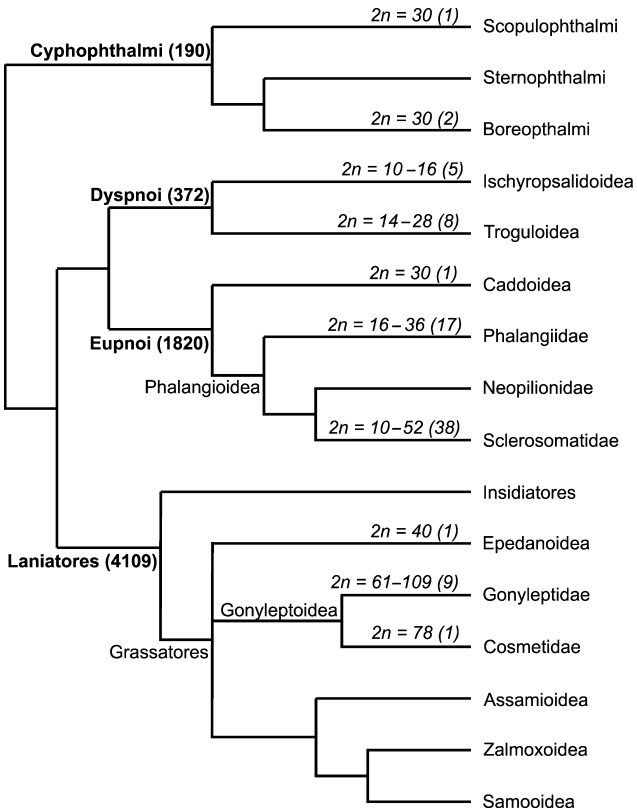

Fig. 2. Simplified phylogeny with number of chromosomes of Opiliones reflecting results from Giribet *et al*. (2012) in Cyphophthalmi, Giribet *et al*. (2010) in Dyspnoi, Hedin *et al*. (2012) in Dyspnoi, and Sharma and Giribet (2011) in Laniatores. Number of described species in suborders from Kury (2012). The diploid numbers and number of karyotyped species (in brackets) per clades added from Tsurusaki (2007), Schneider *et al*. (2008), Rodríguez-Gil and Mola (2010), and the present study.

Most cytogenetic studies in harvestmen are based on mitotic chromosomes, and therefore, only limited information about the course of meiosis is available. The presence of the prominent heteropycnotic blocks on chromosomes from leptotene to pachytene is here documented for the first time in Cyphophthalmi. These heteropycnotic blocks may represent the centromeric regions as previously documented in, for example, the pseudoscorpion *Geogarypus nigrimanus* (Šťáhlavský *et al*. 2006). The poor quality of the pachytene phase in *A. arcticosa* did not enable us to analyse the position of these knobs more precisely and homology with the centromere position is not supported.

Despite the limited information on meiosis in harvestmen, it seems that they posses a low number of mainly terminal chiasmata (e.g. Schneider *et al*. 2009), and only in large chromosomes may we find two chiasmata (Rodríguez-Gil & Mola 2010) as documented here in *A. arcticosa*. Low numbers of chiasmata are also known in other orders of arachnids like spiders (e.g. Dolejš *et al*. 2011; Stávale *et al*. 2011) and pseudoscorpions (e.g. Šťáhlavský *et al*. 2005, 2009). We could not identify morphologically differentiated sex chromosomes in *A. arcticosa*. Non-differentiated sex chromosomes are

probably the ancestral condition for harvestmen as well as for arachnids in general (Král *et al*. 2008). Morphologically differentiated sex chromosomes (XY and ZW) are currently known in Eupnoi and Dyspnoi (Tsurusaki 1989; Tsurusaki & Cokendolpher 1990), and more specific FISH techniques, as for example CGH between males and females, or specific probes for sex-determining genes (see e.g. Liehr 2009), must be used to test the differentiation of sex chromosomes within other harvestmen lineages in the future.

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