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Meiotic karyotypes and structure of testes in males of 17 species of Psyllidae: Spondyliaspidinae (Hemiptera: Psylloidea) from Australia

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Abstract Chromosome numbers and sex-determining systems of 16 species in 10 genera of Australian Psyllidae: Spondyliaspidinae (Hemiptera: Psylloidea) are presented. This is the first comprehensive karyological study for the Spondyliaspidinae. Karyotypes were: 2n = 11 (10 + X) for males of *Australopsylla* sp., *Boreioglycaspis melaleucae* (Moore), *Cardiaspina albitextura* Taylor, *Creiis* sp., *Platyobria lewisi* Taylor and *Spondyliaspis plicatuloides* (Froggatt); 2n = 9 (8 + X) for *Blastopsylla adnatariae* Taylor, *Blastopsylla moorei* Taylor, *Cardiaspina retator* Taylor, *Cryptoneossa* sp. (near *triangula* Taylor), *Glycaspis brimblecombei* Moore; and 2n = 7 (6 + X) for *Anoeconeossa communis* Taylor, *Anoeconeossa* sp. (*communis* group), *Anoeconeossa* sp. (*fuscipennis* group), *A. unicornuta* Taylor and *Creiis vulgaris* Taylor. The chromosome number 2n = 7 is the lowest recorded for any Psylloidea. The number of testicular follicles and arrangement of spermatocyte cysts (spermatocysts) in the follicles of each species, and of an additional species, *Ctenarytaina* sp. are described. Aspects of the karyology and morphology of the male reproductive system of each of the 17 species in 11 genera of Spondyliaspidinae are discussed in terms of recent shifts in spondyliaspidine classification and of the phylogenetic position of the Spondyliaspidinae within the Psylloidea.

Key words chromosome number, karyology, male reproductive system, Psyllidae, Psylloidea, Spondyliaspididae Spondyliaspidinae.

INTRODUCTION

Approximately 110 species of Psylloidea (Hemiptera) have been karyotyped (Maryańska-Nadachowska *et al*. 1992; Kuznetsova *et al*. 1997a; Matcharashvili & Kuznetsova 1997; Maryańska-Nadachowska *et al*. 2001a). Only one of these, *Ctenarytaina eucalypti* (Maskell) (see *Psylla eucalypti* (Maskell) in Maryańska-Nadachowska *et al*. 1992), belongs to the predominantly Australian Spondyliaspidinae (Psyllidae). In the present study the chromosome numbers and sex determining systems of 16 species in 10 genera of Spondyliaspidinae are presented. For each species the male internal reproductive system is examined and the number of testicular follicles and the arrangement of spermatocyte cysts (spermatocysts) in the follicles are described. Until now the male reproductive system of only two species from two spondyliaspidine genera, *Spondyliaspis* sp. and *Ct. eucalypti,* has been studied (Gł*o*wacka *et al*. 1995). In the only other comprehensive study for the Australian psylloid fauna, the karyology and description of the male reproductive system of a further 12 species of Psyllidae: Acizziinae, Carsidaridae and Triozidae from Australia are presented in Maryańska-Nadachowska *et al*. (2001a).

The systematic classification of the world fauna of the Psylloidea has undergone considerable changes and has yet to be determined with certainty. Schwarz (1898) erected a new subfamily of the Psyllidae, the Spondyliaspinae (sic) to accommodate the genus *Spondyliaspis* Signoret. The subfamily was formally defined by Heslop-Harrison (1954). Klimaszewski (1964) raised the taxon to family level and Becker-Migdisova (1973) subdivided it into the predominantly lerp-forming Australian Spondyliaspidinae (to include the genera *Cardiaspina* Crawford, *Creiis* Scott, *Ctenarytaina* Ferris and Klyver, *Eucalyptolym*a Froggatt, *Glycaspis* Taylor, *Phellopsylla* Taylor and *Spondyliaspis* (White & Hodkinson 1985)) and the predominantly American Pachypsyllinae. Spondyliaspidine psyllids are considered either as an independent family, Spondyliaspididae (Becker-Migdisova 1973; Morgan 1984; White & Hodkinson 1985) or as one of the subfamilies of the Psyllidae (Burckhardt 1987; Taylor 1990; Burckhardt 1991; Carver *et al*. 1991).

On the basis of predominantly nymphal characters, White and Hodkinson (1985) included the Euphalerinae, Pachypsyllinae and Spondyliaspidinae in the Spondyliaspididae. They recognised the Diaphorininae and Euphyllurinae as subfamilies of the Aphalaridae but transferred the proposed new tribe, Ctenarytainini, from the Spondyliaspididae to the aphalarid Euphyllurinae. Taylor (1985, 1987a), in recognising their affinities with the Ctenarytainini, placed the new genera *Blastopsylla* Taylor, *Anoeconeossa* Taylor and

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Leptospermonastes Taylor in the Euphyllurinae and *Platyobria* Taylor (Taylor 1987b) in the Diaphorininae (Table 1).

Since then Burckhardt (1987) has synonymised the Spondyliaspididae and Aphalaridae with the Psyllidae without clarifying the position of the subfamilies Euphyllurinae, Euphalerinae and Spondyliaspidinae. Taylor (1990) formalised the tribal classification, recognising these and Ctenarytainini (*sensu* White & Hodkinson 1985) as tribes of the Spondyliaspidinae.

Species studied here belong only to the tribes Ctenarytainini and Spondyliaspidini (*sensu* Taylor 1990). The nymphs of Spondyliaspidini are characterised by the lack of a caudal plate and a circum-anal pore and by the presence of simple setae on the margin of the abdomen. Of the genera of the Spondyliaspidini, those included in our study are the lerp-forming genera, *Australopsylla* Tuthill and Taylor, *Cardiaspina*, *Creiis*, *Glycaspis* and *Spondyliaspis*. The nymphs of Ctenarytainini are characterised by the presence of a caudal plate and complex circum-anal pore fields and modified setae associated with the excretion of white flocculent waste material. Of the genera of the Ctenarytainini, included in the present study are *Anoeconeossa*, *Blastopsylla*, *Cryptoneossa* Taylor and *Ctenarytaina*. *Blastopsylla* are mostly free-living on the meristematic shoot tips of species of *Eucalyptus* and other myrtaceous hosts. *Blastopsylla adnatariae* Taylor, however, form lerps within the rim of the fruits of its host (Taylor 1985). *Ctenarytaina* are free-living on meristematic shoot tips of *Eucalyptus*, and nymphs of *Anoeconeossa* and *Cryptoneossa* live in cryptic situations, often between leaves tied by microlepidoptera, within leaf rolls caused by *Australopsylla* or other gall-formers, or under the vacated lerps of other Spondyliaspidinae.

Of the other two genera studied here, the tribal classification of *Platyobria* remained unclear (Taylor 1987b), and *Boreioglycaspis* Moore was raised from subgeneric rank (from *Glycaspis* – Spondyliaspidini) to generic rank by Burckhardt (1991).

On the basis of definitive adult and nymphal characters Burckhardt (1991) synonymised the Ctenarytainini with the Spondyliaspidini. He included in the Spondyliaspidini the genera *Phellopsylla* and *Phyllolyma* Scott (from Euphalerini) and *Platyobria* (from Aphalaridae: Diaphorininae) (Table 1). He argued that the former two genera differed from the Euphalerini (*sensu* White & Hodkinson 1985) on the basis of

Table 1 Classification of psylloid genera currently placed in Spondyliaspidinae: Spondyliaspidini

After White & Hodkinson (1985) and Taylor (1987a,b)	After Taylor (1990)	After Burckhardt (1991)
Aphalaridae	Psyllidae	Psyllidae
Euphyllurinae	Spondyliaspidinae	Spondyliaspidinae
Ctenarytainini	Ctenarytainini	Spondyliaspidini
**Anoeconeossa* Taylor 1987	*Agelaeopsylla* Taylor 1990	*Agelaeopsylla* Taylor 1990
**Blastopsylla* Taylor 1985	**Anoeconeossa* Taylor 1987	**Anoeconeossa* Taylor 1987
**Ctenarytaina* Ferris & Klyver 1932 (some species referred to *Eucalyptolyma*)	**Blastopsylla* Taylor 1985	**Australopsylla* Tuthill & Taylor 1955
Eurhinocola Crawford 1911	**Cryptoneossa* Taylor 1990	**Blastopsylla* Taylor 1985
Leptospermonastes Taylor 1987	**Ctenarytaina* Ferris & Klyver 1932	**Boreioglycaspis* Moore 1964
Syncarpiolyma Froggatt 1901	*Eriopsylla* Froggatt 1901	**Cardiaspina* Crawford 1911
	Leptospermonastes Taylor 1987	**Creiis* Scott 1882
Diaphorininae	*Syncarpiolyma* Froggatt 1901	**Cryptoneossa* Taylor 1990
Diaphorinini		**Ctenarytaina* Ferris & Klyver 1932
**Platyobria* Taylor 1987	Euphalerini	*Dasypsylla* Froggatt 1900
	Phellopsylla Taylor 1960	*Eriopsylla* Froggatt 1901
Spondyliaspididae	*Phyllolyma* Scott 1882 (= *Cometopsylla* Froggatt 1900)	*Eucalyptolyma* Froggatt 1901
Euphalerinae		*Eurhinocola* Crawford 1911
Phellopsylla Taylor 1960	Spondyliaspidini	**Glycaspis* Taylor 1960
Phyllolyma Scott 1882 (= *Cometopsylla* Froggatt 1900)	**Australopsylla* Tuthill & Taylor 1955	*Hyalinaspis* Taylor 1960
	**Cardiaspina* Crawford 1911	*Kenmooreana* Taylor 1984
Spondyliaspidinae	**Creiis* Scott 1882	*Lasiopsylla* Froggatt 1900
**Australopsylla* Tuthill & Taylor 1955	*Dasypsylla* Froggatt 1900	*Leptospermonastes* Taylor 1987
**Cardiaspina* Crawford 1911	*Eucalyptolyma* Froggatt 1901	*Phellopsylla* Taylor 1960
**Creiis* Scott 1882	*Eurhinocola* Crawford 1911	*Phyllolyma* Scott 1882 (= *Cometopsylla* Froggatt 1900)
Dasypsylla Froggatt 1900	**Glycaspis* Taylor 1960	**Platyobria* Taylor 1987
Eriopsylla Froggatt 1901	*Hyalinaspis* Taylor 1960	**Spondyliaspis* Signoret 1879
Eucalyptolyma Froggatt 1901	*Kenmooreana* Taylor 1984	*Syncarpiolyma* Froggatt 1901
**Glycaspis* Taylor 1960	*Lasiopsylla* Froggatt 1900	
Hyalinaspis Taylor 1960	**Spondyliaspis* Signoret 1879	
Kenmooreana Taylor 1984		
Lasiopsylla Froggatt 1900	Unplaced	
**Spondyliaspis* Signoret 1879	**Platyobria* Taylor 1987	

*Represented in the present study.

nymphal characters and that the similarity of *Platyobria* to Diaphorininae was superficial. He concluded that the Ctenarytainini (*sensu* White & Hodkinson 1985; Taylor 1990) was unlikely to be supported phylogenetically, arguing that the presence of a caudal plate and nymphal lanceolate setae (characteristic of the Ctenarytainini; Taylor 1990) was plesiomorphic and suggested that lerp-forming evolved several times independently. Clearly the phylogenetic relationship between spondyliaspidine genera requires further clarification.

MATERIALS AND METHODS

All species for the present study were collected and identified (by GST) in Australia. For cytological studies, specimens of adult males were dropped live into freshly prepared Carnoy's fixative (25% glacial acetic acid in ethanol). The locations of collection sites and sample sizes are given in Table 2.

Cytogenetic studies were carried out (by AM and VGK). Pretreatment, preparation of slides and staining of chromosome were made according to the protocol described by Maryańska-Nadachowska *et al*. (1992).

To study the male internal reproductive system, the same material fixed for karyological studies was used. Specimens were dissected in a drop of fixative and the reproductive system was transferred to a drop of 45% acetic acid on a microscope slide for examination. The testicular follicles were carefully separated from each other, then a follicle was transferred to a small drop of 45% acetic acid on another slide and carefully covered by a coverslip. The pattern of spermatocyst arrangement within a follicle was observed under a light stereomicroscope.

The karyology of a total of 16 species belonging to 10 genera of Australian Psyllidae: Spondyliaspidinae and the internal reproductive system of an additional species were examined.

Voucher specimens, slide material mounted in Gurr's DePex mounting medium (by the method in Taylor 1999), specimens preserved in 70% ethanol, and specimens used in cytogenetic studies (in Carnoy's fixative), have been deposited in the Waite Insect and Nematode Collection, Adelaide University.

RESULTS

Karyotypes

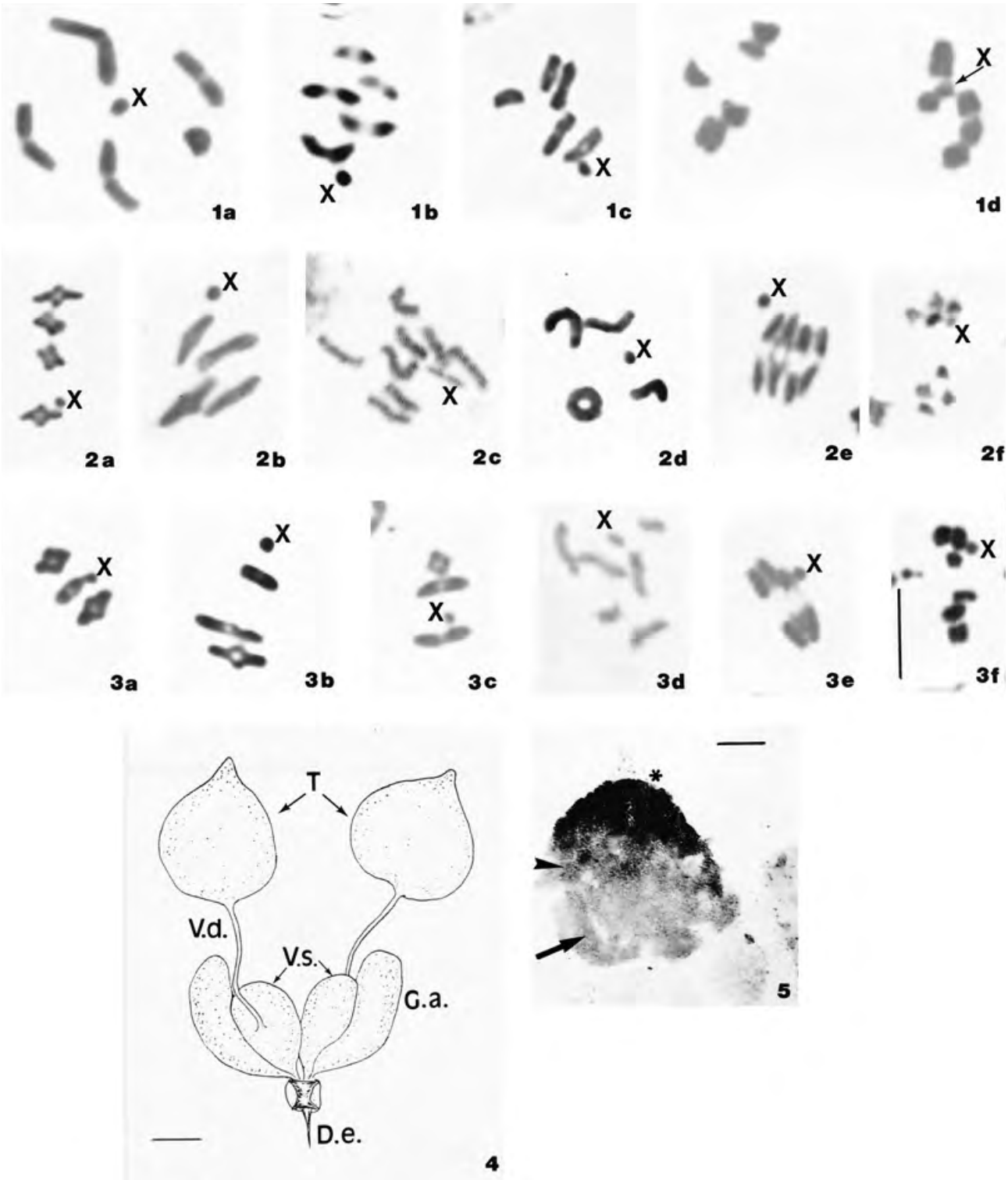
Of the 16 species examined, chromosome numbers were either 11, 9 and 7 in male diploid karyotypes (Table 2).

In six species, belonging to six genera, the karyotype of 2n = 11 (10 + X) was found in males. This karyotype appeared to be characteristic of *Australopsylla* sp., *Boreioglycaspis melaleucae* (Moore), *Cardiaspina albitextura* Taylor, *Creiis* sp., *Platyobria lewisi* Taylor, and *Spondyliaspis plicatuloides* (Froggatt). In diakinesis and in metaphase I (MI), five chiasmatic bivalents and an univalent X-chromosome were observed (Fig. 1a–c; $n = 5 + X$). The meiotic karyotypes appeared to be principally similar in

Table 2 Species of Australian Psyllidae: Spondyliaspidinae with host plant, collection data and number of specimens examined, number of chromosomes (2n) and number of follicles per testis

Species	Host site	Collection site and date (number examined)	No. chomosomes 2n (metaphase I)	No. follicles per testis
Anoeconeossa communis Taylor 1987	*Eucalyptus camaldulensis*	SA, Ambleside (near Hahndorf), 4.v.1997 (6 males)	6 + X	1
Anoeconeossa unicornuta Taylor 1987	*Eucalyptus leucoxylon*	SA, Adelaide, Urbrae, Waite Campus, 24.x.1997 & 4.xi.1997 (5 males)	6 + X	1
Anoeconeossa sp. (*communis* group Taylor 1987a)	*Eucalyptus leucoxylon*	SA, Adelaide, Urbrae, Waite Campus, 24.x.1997 & 4.xi.1997 (5 males)	6 + X	1
Anoeconeossa sp. (*fuscipennis* group Taylor 1987a)	*Eucalyptus leucoxylon*	SA, Adelaide, Urbrae, Waite Campus, 24.x.1997 & 4.xi.1997 (5 males)	6 + X	1
Australopsylla sp.	*Eucalyptus* sp. from white bivalve lerps in curled leaf galls	SA, Strathalbyn-Goolwa, 18.xii.1998 (4 males)	10 + X	1
Blastopsylla adnatariae Taylor 1985	*Eucalyptus lehmannii*	SA, Adelaide, Urbrae, Waite Campus, 22.ix.1997 (8 males)	8 + X	1
Blastopsylla moorei Taylor 1985	*Melaleuca lanceolata*	SA, Goolwa, 29.x.1997 (7 males)	8 + X	1
Boreioglycaspis melaleucae (Moore 1964)	*Melaleuca quinquenervia*	Qld, Brisbane, Indooroopilly, CSIRO labs, 27.vii.1999 (5 males)	10 + X	1
Cardiaspina albitextura Taylor 1962	*Eucalyptus camaldulensis*	SA, Goolwa, 29.x.1997 (5 males)	10 + X	1
Cardiaspina retator Taylor 1962	*Eucalyptus camaldulensis*	SA, Ambleside (near Hahndorf), 4.v.1997 (10 males)	8 + X	1
Creiis sp.	*Eucalyptus leucoxylon*	SA, Adelaide, Urbrae, Waite Campus, 24.x.1997 (4 males)	10 + X	1
Cryptoneossa vulgaris Taylor 1990	*Eucalyptus leucoxylon*	SA, Adelaide, Urbrae, Waite Campus, 24.x.1997 & 4.xi.1997 (4 males)	6 + X	1
Cryptoneossa sp. (near *triangula* Taylor 1990)	*Angophora floribunda*	SA, Adelaide, Urbrae, Waite Campus, 22.ix.1997 (5 males)	8 + X	1
Ctenarytaina sp.	*Melaleuca lanceolata*	SA, Goolwa, 29.x.1997 (2 males)	–	1
Glycaspis brimblecombei Moore 1964	*Eucalyptus camaldulensis*	SA, Ambleside (near Hahndorf), 4.v.1997 (13 males)	8 + X	1
Platyobria lewisi Taylor 1987	*Eucalyptus camaldulensis*	SA, Ambleside (near Hahndorf), 30.x.1998 (7 males)	10 + X	1
Spondyliaspis plicatuloides (Froggatt 1990)	*Eucalyptus microcarpa*	SA, Adelaide, Urbrae, Waite Campus, 26.viii.1999 (1 male)	10 + X	1

All collected by GST. Qld, Queensland; SA, South Australia.



Figs 1–5. Chromosomes and male reproductive systems of Australian Spondyliaspidinae. **(1a–c)** First metaphases. **(1a)** *Cardiaspina albitextura*; **(1b)** *Platyobria lewisi*; **(1c)** *Creiis* sp. **(1d)** second metaphases of *Ca. albitextura*, two daughter cells (5 + 0 and 5 + X). **(2a,b)** First metaphases. **(2a)** *Blastopsylla adnatariae*; **(2b)** *Bl. moorei*; **(2c)** spermatogonial mitotic metaphase of *Bl. adnatariae*; **(2d)** first metaphase of *Ca. retator*, one bivalent with two chiasmata; **(2e)** first anaphase of *Bl. adnatariae*; **(2f)** second metaphases of *Bl. adnatariae*, two daughter cells (4 + 0 and 4 + X). **(3a–c)** First metaphases. **(3a)** *Anoeconeossa unicornuta*; **(3b)** *Cryptoneossa vulgaris*; **(3c)** *A. communis*; **(3d)** spermatogonial mitotic metaphase of *Anoeconeossa* sp. (*communis* group); **(3e)** first anaphase of *Anoeconeossa* sp. (*fuscipennis* group); **(3f)** second metaphases of *A. unicornuta*, two daughter cells (3 + 0 and 3 + X). **(4)** Male reproductive system of *P. lewisi* (abbreviations: D.e., ductus ejaculatorius; G.a., glandulae accessoria; T, testes; V.d., vasa deferentia; V.s., vesiculae seminalis). **(5)** Testicular follicle of *P. lewisi* with three zones of differentiation: premeiotic divisions (asterisk), meiotic divisions (arrow head), spermiogenesis (arrow). Figures 1–3 scale = 10 μm; Figs 4,5, scale = 100 μm.

structure. In these karyotypes autosomal bivalents consecutively decreased in size. The X-chromosome was the smallest element of the set, being a little smaller than the smallest half-bivalent. In MI the sex univalent tended to be placed at the periphery of the metaphase plate. Bivalents showed a single chiasma each, the chiasmata being mainly located terminally. Two types of metaphase II (MII) were observed, those with five autosomes and those with five autosomes and an additional X-chromosome (Fig. 1d).

In five species belonging to four genera, the karyotype of 2n = 9 (8 + X) was found in males. This karyotype appeared to be characteristic of *Blastopsylla adnatariae* Taylor, *Bl. moorei* Taylor, *Ca. retator* Taylor, *Cryptoneossa* sp. (near *triangula* Taylor) and *Glycaspis brimblecombei* Moore. In diakinesis and in MI, four chiasmatic bivalents and an univalent X-chromosome were observed (Fig. 2a–c; $n = 4 + X$). Karyotypes appeared to be similar in structure. All bivalents constituted a row consecutively decreasing in size. The X-chromosome was the smallest element of the set and tended to be placed at the periphery of the metaphase plate. The aforementioned size structure of the karyotype was also observed in the mitotic metaphases in *B. adnatariae*. Figure 2(c) shows the mitotic metaphase of this species, in which all chromosomes, including the X, are of rather similar size, and the X-chromosome being well distinguished from the autosomes due to its negative heteropycnosis. We observed that the sex chromosome displayed a rod-shaped form at this stage, whereas it was always of a rounded form in diakinesis and MI. In MI, every bivalent was held together by one terminal or subterminal chiasma, except in *C. retator* where one bivalent showed two chiasmata in a part of the nucleus (Fig. 2d). Two types of MII could be found; those with four autosomes and those with four autosomes and the X-chromosome (Fig. 2e,f).

In five species belonging to two genera the karyotype of 2n = 7 (6 + X) was found in males. This karyotype appeared to be characteristic of all species of the genus *Anoeconeossa*; *A. communis* Taylor, *Anoeconeossa* sp. (*communis* group), *Anoeconeossa* sp. (*fuscipennis* group), and *A. unicornuta* Taylor, as well as *Cryptoneossa vulgaris*. In diakinesis and in MI we could see three chiasmatic bivalents and a univalent X-chromosome (Fig. 3a–c; $n = 3 + X$). The karyotypes of the aforementioned species appeared similar in structure. Bivalents were similar in size but both the largest and the smallest were readily distinguished. The X-chromosome was approximately half the size of the smallest half-bivalent and tended to be placed at the periphery of the metaphase plate. The aforementioned size structure of the karyotype was confirmed in the mitotic metaphases, found in *Anoeconeossa* sp. (*communis* group) (Fig. 3d). At this stage the X-chromosome was of the rod form, although it exhibited a rounded form in diakinesis and MI. In MI each bivalent displayed a single chiasma, which was mainly terminal or subterminal, while an interstitial chiasma was also observed in one bivalent of *Anoeconeossa* sp. (*communis* group) (Fig. 3c). As a result of anaphase I (AI), one daughter nucleus in MII displayed three autosomes, while in the other three autosomes plus the X-chromosome were observed (Fig. 3e,f).

We found that two of the 10 genera studied had species with variable numbers of chromosomes in their karyotypes. These were *Cardiaspina*, with *C. albitextura* with 2n = 11 (10 + X) and *C. retator* with 2n = 9 (8 + X), and *Cryptoneossa*, with *Cryptoneossa* (near *triangula*) with 2n = 9 (8 + X) and *Cr. vulgaris* Taylor with 2n = 7 (6 + X).

Male reproductive system and arrangement of spermatocysts in follicles

The male internal reproductive system is principally similar in the species studied. Males displayed paired testes, each with a relatively long seminal duct (vas deferens). The vasa deferentia are provided with well-defined seminal vesicles (vesiculae seminales), which are fairly large in the mature males. In front of the sperm pump the seminal ducts enter the ejaculatory duct (ductus ejaculatorius) in proximity to paired accessory glands (glandulae accessoria; Fig. 4). The accessory glands are similar in shape to seminal vesicles with a slightly elongated form. Each testis consists of a single follicle. The follicle is oval in form with a slightly elongate apex, its length not much exceeding its width. In young males the follicles exhibit three poorly distinguished zones of differentiation: the germarium (premeiotic divisions); the zone of growth (meiotic divisions); and the zone of spermiogenesis (Fig. 5). Within the meiotic zone, spermatocysts are arranged without any apparent alignment. An absence of alignment is also characteristic of the zone of spermiogenesis, in which maturing spermatids and mature sperms are randomly distributed.

DISCUSSION

Karyotypes

During the last decade (as a result of intensive karyotype investigations) approximately 125 species (including 16 presented here) from 51 psylloid genera have been studied (Maryańska-Nadachowska *et al*. 1992; Kuznetsova *et al*. 1997a; Matcharashvili & Kuznetsova 1997; Maryańska-Nadachowska *et al*. 2001a). These figures include all families except Phacopteronidae. Diploid chromosome numbers of Psylloidea lie between 7 and 26 in males with a marked mode at 25. This number has been found in every family studied, including the most primitive taxa, and covers approximately 70% of all karyotyped species. A sex chromosome system of X0 in males occurs in the overwhelming majority (approximately 95%) of all species studied. Thus, the formula of the modal karyotype is 2n = 25 (24 + X) in males and 2n = 26 (24 + XX) in females. It is reasonable to suggest that the ancestral psylloid possessed this karyotype (Kuznetsova *et al*. 1995, 1997a; Matcharashvili & Kuznetsova 1997) and it could be argued that all other karyotypes in the Psylloidea could have evolved as derived characters in different families.

The only known chromosome number that exceeds the putative ancestral state is 2n = 26, recently found in the

genus *Bactericera* Puton (Triozidae), in which a group of species has acquired a Y-chromosome during its evolution (Kuznetsova *et al*. 1997a; Maryańska-Nadachowska *et al*. 2001b). All other derivative numbers are lower than this mode, strongly suggesting that chromosome fusions have predominated during the evolution of the Psylloidea. In the majority of known cases few fusions have occurred, resulting in insignificant differences in chromosome numbers between related species. One of the exceptions is in the genus *Trioza* Förster (Triozidae). In *Trioza ilicina* (de Stefani Perez) and *T. remota* Förster the karyotype is 2n = 15 (14 + X) in males (Maryańska-Nadachowska & Hodkinson 1993; Maryańska-Nadachowska *et al*. 1996). This suggests the occurrence of 10 autosome fusions during their evolution because all other karyotyped species of *Trioza* have retained the ancestral karyotype of 2n = 25. Recently Matcharashvili and Kuznetsova (1997) reported 2n = 13 (12 + X) for *Craspedolepta bulgarica* Klimaszewski (Aphalaridae), whereas 2n = 25 has been elsewhere reported for seven other representatives of this genus. *Psylla foersteri* Flör constitutes the most intriguing example. This species displays 15 chromosomes in males, including one huge autosome pair, which may have arisen by multiple fusions of autosomes from an ancestral karyotype of 2n = 25 (Suomalainen & Halkka 1963). It remains unclear as to why the ancestral autosomes fused into one large linkage group in *Ps. foersteri*, whereas in all other species with reduced chromosome numbers the neochromosomes show a much lower range of size differences. These observations would indicate an accidental establishment of chromosome fusions in the karyotype evolution of the Psylloidea.

Until the present study, the lowest chromosome numbers recorded for the Psylloidea was in the Rhinocolinae (Aphalaridae). *Agonoscena cisti* (Puton), *A. targionii* (Lichtenstein) and *Lisronia varicicosta* (Hodkinson & Hollis) each have 2n = 13, and *Rhinocola aceris* (L.) has 2n = 11 (Maryańska-Nadachowska *et al*. 1992; Maryańska-Nadachowska & Hodkinson 1993).

Our data suggest that the Spondyliaspidinae is one further such taxon of the Psylloidea characterised by consistently low chromosome numbers. Indeed, 2n = 11 is shared with only one other psylloid studied, *R. aceris*; the remaining spondyliaspidine genera studied here display diploid chromosome numbers of 2n = 9 and 2n = 7, being the lowest recorded for any Psylloidea. These karyotypes indicate that the Spondyliaspidinae is a considerably derived group within the superfamily.

According to the classification of White and Hodkinson (1985), Spondyliaspidinae is included in the family Spondyliaspididae together with three other subfamilies: the Arepuniinae, Euphalerinae and Pachypsyllinae. Chromosome data are currently available for three, including the Euphalerinae (Park *et al*. 1995), Pachypsyllinae (Maryańska-Nadachowska & Yang 1997) and Spondyliaspidinae (present study). In the Euphalerinae, the only species studied was *Euphalerus robinae* with 2n = 25 (24 + X) in males. In the Pachypsyllinae, from nine species studied, 2n = 25 was clearly predominant. In the evolution of Pachypsyllinae 2–4 autosome fusions, respectively, from an ancestral state of 25, have resulted in 2n = 23 (22 + X) in *Pachypsylla venusta* (Osten-Sacken) and 2n = 21 (20 + X) in *Pa. celtidisgemma* Riley.

Karyotypes of Spondyliaspidinae suggest a common origin, with 2n = 11 (10 + X) probably being the ancestral state. A karyotype of 2n = 11 was observed in six of 16 species from six of 10 genera studied, indicating 14 autosome fusions early in the evolution of the Spondyliaspidinae, from the ancestral psylloid karyotype of 2n = 25. A further two and two additional fusions, respectively, were necessary to reduce the chromosome number from 11 to 9 and 7. There are some indications that these derived characters may have evolved independently in different taxa within the Spondyliaspidinae, given that two genera, *Cardiaspina* and *Cryptoneossa*, had species with variable chromosome numbers (e.g. *Ca. albitextura* possess 2n = 11 and *Ca. retator* had 2n = 9, and *Cryptoneossa* (near *triangula*) possesses 2n = 9 and *Cr. vulgaris* had 2n = 7). All spondyliaspidine species studied here displayed an X0 sex chromosome system.

Although it is plausible to suggest the monophyletic origin of the low chromosome numbers in the Spondyliaspidinae (with an inferred ancestral state of 2n = 11), interesting observations on the phylogeny of the group could be made by comparing chromosome numbers within genera referred to each of the classifications given by Taylor (1990) and Burckhardt (1991) of the spondyliaspidine Psylloidea.

Taylor (1990) recognised four tribes (Ctenarytainini, Euphalerini, Euphyllurini and Spondyliaspidini). Two of these are represented in the present study. Of the Ctenarytainini, the genera *Anoeconeossa* had a karyotype of 2n = 7 in the four species studied, *Cryptoneossa* had 2n = 7 and 2n = 9 in two species studied, respectively, and *Blastopsylla* had 2n = 9 in two species studied. Uncharacteristically, however, the genus *Ctenarytaina* showed 2n = 21 (20 + X) in one previously studied species (Maryańska-Nadachowska *et al*. 1992). *Ctenarytaina eucalypti* displayed a karyotype closer to the ancestral state (2n = 24 + X) compared with three other genera of the Ctenarytainini (*Anoeconeossa*, *Blastopsylla* and *Cryptoneossa*), of which *Anoeconeossa* constitutes the most derived condition.

Of the Spondyliaspidini, the genera *Cardiaspina* had a chromosome number of 2n = 7 and 2n = 9 in the two species studied, respectively; *Glycaspis* had 2n = 9 in one species studied, and *Australopsylla*, *Creiis* and *Spondyliaspis* had 2n = 11 in the single species of each studied. Thus the Ctenarytainini had genera predominantly with karyotypes of 2n = 7 and 2n = 9, and the Spondyliaspidini had karyotypes predominantly 2n = 9 and 2n = 11. While this tends to support the tribal classification of Taylor (1990), it suggests an independent reduction of chromosome numbers at least once in the Ctenarytainini, and at least once in the Spondyliaspidini.

Male reproductive system and arrangement of spermatocysts in follicles

The male internal reproductive system is similar in all psylloid species studied (Gℓowacka 1983; Gℓowacka & Maryańska-Nadachowska 1993). We confirm this for the Spondyliaspidinae. The system consists of a pair of testes, a pair of seminal vesicles, a pair of accessory glands and a single ejaculatory duct.

Variable within the Psylloidea are the number and form of testicular follicles and arrangement of spermatocysts within a follicle (Gℓowacka *et al*. 1995). The number of follicles per testes range from one to five. The two-follicular state occurs most commonly throughout the Psylloidea, having been found in 117 of 166 species (approximately 65% of all species studied), including the most primitive taxa. Therefore it is most likely that this state was ancestral in the evolution of Psylloidea, and that variance from this indicates a derived condition that has apparently evolved independently in different families (Kuznetsova *et al*. 1997b).

In different taxa, follicles may show differences in form and pattern of the internal spermatocyst arrangement. A great majority of species display follicles of elongate form; a character that appears to be stable over the higher taxa (upper classification) of the Psylloidea. In some species, however, follicles are clearly shorter and display an oval form, which can be observed both in the young and in adult males. Within a follicle, spermatocysts are mainly aligned in one, two or several rows (Gℓowacka *et al*. 1995). In some species, however, spermatocysts show a random distribution, their outline often difficult to distinguish (Gℓowacka & Maryańska-Nadachowska 1998; present study). This arrangement appears to be characteristic of all species with short oval follicles such as *Ct. eucalypti* (Gℓowacka *et al*. 1995) and *Mycopsylla fici* (Tryon) (Gℓowacka & Maryańska-Nadachowska 1998), and for all species studied here.

These studies indicate a taxonomic and phylogenetic significance to the number of testicular follicles (characters that exhibit stability within the higher taxa), showing them as reliable synapomorphies. Taxa that possess more than two follicles per testis are the genera *Psylla* Geoffroy *s. str.*, *Cyamophila* Loginova and *Homotoma* Guérin-Méneville (Gℓowacka *et al*. 1995; Kuznetsova *et al*. 1997b). One follicle per testis constitutes a character consistent within some subfamilies: for example, in the Rhinocolinae (Aphalaridae) with all six genera studied (*Leurolophus* Tuthill, *Rhinocola* Förster, *Agonoscena* Enderlein, *Lisronia* Loginova, *Megagonoscena* and *Tainarys* Bréthes); in Carsidarinae (Carsidaridae) with all three genera studied (*Tenaphalara* Kuwayama, *Mesohomotoma* Kuwayama, and *Paracarsidara* Heslop-Harrison); and in Pachypsyllinae (the sister group of Spondyliaspidinae (Burckhardt 1991)) with both genera studied (*Pachypsylla* Riley and *Tetragonocephala* Crawford).

The present study suggests that one follicle per testis is a plesiomorphic character within the Spondyliaspidinae, occurring in all 17 species referred to all 11 spondyliaspidine genera (Table 2). But one exception to this configuration in the Spondyliaspidinae is where two follicles per testis have been reported for *Spondyliaspis* sp. (Gℓowacka *et al*. 1995). This may indicate that one follicle per testis may constitute the ancestral character for Spondyliaspidinae and Pachypsyllinae and that two follicles represents the derived condition, having apparently evolved once in the Spondyliaspidinae. Other closely related taxa are yet to be studied.

Follicle number per testis may be used to define certain taxa. In the classification of White and Hodkinson (1985), the new tribe Ctenarytainini was placed together with Euphyllurini in the Euphyllurinae (Aphalaridae). In the classification by Taylor (1990) and following the synonymy of the Spondyliaspididae and Aphalaridae with the Psyllidae (Burckhardt 1987), the Euphyllurini and Ctenarytainini were transferred to the Spondyliaspidinae. Consistent with the classification by Taylor (1990) is that two species of Ctenarytainini, *Ct. eucalypti* (Gℓowacka *et al*. 1995) and *Ctenarytaina* sp. (present study) (referred to the Spondyliaspidinae) both possess one follicle per testis, whereas two species of *Euphyllura* Förster (*E. olivina* Costa and *E. phillyreae* Förster) display two follicles per testis (Gℓowacka 1983). A similarity in the testis structure of *Ctenarytaina* and the genera *Anoeconeossa, Blastopsylla* and *Cryptoneossa* supports the monophyly of the Ctenarytainini even though chromosome numbers between *Ctenarytaina* and the other genera are widely disparate.

Based on this classification we suggest that oligomerisation of the follicle number may have occurred in a common ancestor of the Spondyliaspidinae and Pachypsyllinae or in each of these groups independently. Species in the genus *Euphyllura* and one of the two species of *Spondyliaspis* studied (Gℓowacka *et al*. 1995) appear to have retained the ancestral state of this character or, in the latter, two follicles may represent a secondarily derived condition from one follicle per testis, as found in all other spondyliaspidines studied.

Evidence presented supports the suggestion that karyotype of 2n = 25 (24 + X), and testes consisting of two follicles each, constitute the ancestral characters in the Psylloidea (Maryańska-Nadachowska *et al*. 1992; Kuznetsova *et al*. 1997a,b). For the Spondyliaspididae (*sensu* White & Hodkinson 1985) (Psyllidae: Spondyliaspidinae (*sensu* Burckhardt 1987; Taylor 1990)), strong reduction of chromosome numbers by numerous autosomal fusions, and oligomerisation of the number of seminal follicles have occurred in the evolution of the group. Derived character states exhibit considerable stability, being susceptible to few secondary polymerisation processes. In summary we consider the Australian Spondyliaspidinae as a compact, advanced and specialised taxon that has evolved very intensive rearrangements of its genetic structure in its evolution.

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