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

Anna Maryańska-Nadachowska,1 Gary S Taylor2,* and Valentina G Kuznetsova3

1Department of Experimental Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Śląskowska 17, PL 30–016 Kraków, Poland.
2Department of Applied and Molecular Ecology, Waite Campus, Adelaide University, PMB 1, Glen Osmond, SA 5064, Australia.
3Department of Karyosystematics, Zoological Institute, Russian Academy of Sciences, 199034 St Petersburg, Russia.

Abstract
Chromosome numbers and sex-determining systems of 16 species in 10 genera of Australian Psyllidae: Spondyliaspidae (Hemiptera: Psylloidea) are presented. This is the first comprehensive karyological study for the Spondyliaspidae. Karyotypes were: 2n = 11 (10 + X) for males of Australopsylla sp., Boreioglycaspis melaleucae (Moore), Cardiaspina albitextura Taylor, Creis sp., Platyobria lewisi Taylor and Spondyliaspis plicatuloides (Froggatt); 2n = 9 (8 + X) for Blastopsylla adnatae Taylor, Blastopsylla moorei Taylor, Cardiaspina retator Taylor, Cryptoneossa sp. (near trianguula 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 Creis 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 Spondyliaspidae are discussed in terms of recent shifts in spondyliaspidine classification and of the phylogenetic position of the Spondyliaspidae within the Psylloidea.

Key words chromosome number, karyology, male reproductive system, Psyllidae, Psylloidea, Spondyliaspidae

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. 2001a). One only of these, Ctenarytaina eucalypti (Maskell) (see Psylla eucalypti Maskell in Maryańska-Nadachowska et al. 2002), belongs to the predominantly Australian Spondyliaspidae (Psylloidea). In the present study the chromosome numbers and sex determining systems of 16 species in 10 genera of Spondyliaspidae 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 is examined and 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 Spondyliaspidae are discussed in terms of recent shifts in spondyliaspidine classification and of the phylogenetic position of the Spondyliaspidae within the Psylloidea.

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 Spondyliaspidae (to include the genera Cardiaspina Crawford, Creis Scott, Ctenarytaina Ferris and Klyver, Eucalyptolyma Froggatt, Glycaspis Taylor, Phelopsylla Taylor and Spondyliaspis (White & Hodkinson 1985)) and the predominantly American Pachy-psyllinae. Spondyliaspidean psyllids are considered either as an independent family, Spondyliaspidae (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, Pachy-psyllinae and Spondyliaspidae in the Spondyliaspidae. They recognised the Diaphoriniinae and Euphylurinae as subfamilies of the Aphalaridae but transferred the proposed new tribe, Ctenarytainini, from the Spondyliaspidae to the aphalarid Euphylurinae. Taylor (1985, 1987a), in recognising their affinities with the Ctenarytainini, placed the new genera Blastopsylla Taylor, Anoeconeossa Taylor and

*Author to whom correspondence should be addressed (email: gary.taylor@adelaide.edu.au).
Leptospermonastes Taylor in the Euphyllurinae and Platy-
obria 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 Spondyliaspidiini 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 Spondyliaspidiini, those included in our study are the lerp-forming genera, Australopsylla Tuthill and Taylor, Cardiaspina, Creis, Glycaspis and Spondylaspsis. 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, Crypto-
neossa 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 meris-
tematic shoot tips of Eucalyptus, and nymphs of Anoecone-
nessa and Cryptoneessa 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 Spondyliaspidiini.

Of the other two genera studied here, the tribal classification of Platobbyria 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 Platobbyria (from Aphalaridae: Diaphorininae) (Table 1). He argued that the former two genera differed from the Eupha-
erini (sensu White & Hodkinson 1985) on the basis of

| Table 1 Classification of psylloid genera currently placed in Spondyliaspidinae: Spondyliaspidini |
|-------------------------------------------------------|-----------------|-----------------|
| Aphalaridae                                            | Psyllidae                   | Psyllidae                   |
| Euphyllurinae                                          | Spondyliaspidinae           | Spondyliaspidinae           |
| Ctenarytainini                                         | Ctenarytainini              | Spondyliaspidina            |
| *Anoeconeossa Taylor 1987                              | Agelaepsylla Taylor 1990    | *Anoeconeossa Taylor 1987   |
| *Blastopsylla Taylor 1985                              | *Anoeconeossa Taylor 1987   | *Australopsylla Taylor 1955 |
| *Ctenarytaina Ferris & Klyver 1932                     | *Blastopsylla Taylor 1985   | *Blastopsylla Taylor 1985   |
| (some species referred to Eucalyptolyma)                | *Cryptoneessa Taylor 1990   | *Boreioglycaspis Moore 1964 |
| Eurihocola Crawford 1911                              | *Ctenarytaina Ferris & Klyver 1932| *Cardiaspina Crawford 1911 |
| Leptospermonastes Taylor 1987                          | Eriopsylla Froggatt 1901    | *Creis Scott 1882           |
| Syncarpiozyma Froggatt 1901                            | Leptospermonastes Taylor 1987| *Cryptoneessa Taylor 1990   |
| Diaphorininae                                          | Syncarpiozyma Froggatt 1901 | *Ctenarytaina Ferris & Klyver 1932 |
| Diaphorini                                             | Euphalerini                 | Dassypsylla Froggatt 1900   |
| *Platobbyria Taylor 1987                               | Phellopsylla Taylor 1960    | Eriopsylla Froggatt 1901    |
|                                                                 | Phyllolyma Scott 1882 (= Cometopsylla | Eucalyptolyma Froggatt 1901 |
| Spondyliaspidinae                                      | Froggatt 1900)              | Eurihocola Crawford 1911   |
| Euphalerina                                            | Spondyliaspidina            | *Glycaspis Taylor 1960      |
| *Phellopsylla Taylor 1960                              | *Australopsylla Tuthill & Taylor 1955| Hyalinaspis Taylor 1960 |
| Phyllolyma Scott 1882 (= Cometopsylla                   | *Cardiaspina Crawford 1911 | Kenmooreana Taylor 1984    |
| Froggatt 1900)                                         | *Creis Scott 1882           | Lasopsylla Froggatt 1900    |
| Spondyliaspidinae                                      | Dassypsylla Froggatt 1900   | Leptospermonastes Taylor 1987|
| *Australopsylla Tuthill & Taylor 1955                  | Eucalyptolyma Froggatt 1901| Phellopsylla Taylor 1960   |
| *Cardiaspina Crawford 1911                            | Eurihocola Crawford 1911    | Phyllolyma Scott 1882 (= Cometopsylla | |
| *Creis Scott 1882                                      | *Glycaspis Taylor 1960      | Froggatt 1900)              |
| Dassypsylla Froggatt 1900                              | Hyalinaspis Taylor 1960     | *Platobbyria Taylor 1987    |
| Eriopsylla Froggatt 1901                               | Kenmooreana Taylor 1984     | *Spondylaspsis Signoret 1879|
| *Glycaspis Taylor 1960                                 | Lasiopsylla Froggatt 1900   | Syncarpiozyma Froggatt 1901|
| Hyalinaspis Taylor 1960                                | *Spondylaspsis Signoret 1879| |
| Kenmooreana Taylor 1984                                |                             |                             |
| Lasiopsylla Froggatt 1900                              |                             |                             |
| *Spondylaspsis Signoret 1879                          |                             |                             |

*Represented in the present study.
RESULTS

Karyotypes

Of the 16 species examined, chromosome numbers were either 11 or 7 in multiploid species (Table 2), characteristic of the Spondyliaspidinae and the Diaphorinae. In six species, belonging to six genera (Table 2), the karyotype of 2n = 11 (10 + X) was found in males. This karyotype appeared to be characteristic of the Spondyliaspides (Froggatt). In diapause and in diapause-dyliaspis plicatuloides (Froggatt), Cardiaspina albitextura (Taylor), Crepis sp., Platyobria lewisi (Taylor), and Spondyliaspis plicatuloides (Froggatt), Cardiaspina albitextura (Taylor), Crepis sp., Platyobria lewisi (Taylor), and Spondyliaspis plicatuloides (Froggatt) appeared to be characteristic of Cardiaspina lewisi (Taylor), Crepis sp., Platyobria lewisi (Taylor), and Spondyliaspis plicatuloides (Froggatt). All karyotypes used for karyological studies were deposited in the Waite Insect and Nematode Collection, Adelaide University.

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 (2% acetic acid in ethanol). The locations of collection sites and sample sizes are given in Table 2.

Pretreatment, preparation of slides and staining of chromosomes were performed according to the protocol described by Taylor et al. (1990). To study the male internal reproductive system, the same material used for karyological studies was used. The pattern of spermatocyst arrangement within a follicle was observed under a light stereomicroscope. To study the male internal reproductive system, the same material used for karyological studies was used. The pattern of spermatocyst arrangement within a follicle was observed under a light stereomicroscope.

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Figs 1–5. Chromosomes and male reproductive systems of Australian Spondyliaspidinae. (1a–c) First metaphases. (1a) Cardiaspina albitextura; (1b) Platysoria 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 consecu-
tively 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 ter-
minally. 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 adnataeae Taylor, Bl. moorei
Taylor, Ca. retator Taylor, Cryptoneossa sp. (near triangula
Taylor) and Glycaspis brimblecombii Moore. In diakinesis
and in MI, four chiasmatic bivalents and an univalent X-
chromosome were observed (Fig. 2a-c; n = 4 + X). Karyo-
types 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 afore-
mentioned size structure of the karyotype was also observed in
the mitotic metaphases in A. unicornuta. 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-chromo-
some being well distinguished from the autosomes due to its
negative heteropycnosis. We observed that the sex chromo-
some 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 Anoeconeossa; A. communis Taylor, Anoeconeossa sp. (communis group),
Anoeconeossa sp. (fuscinipennis 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. Bival-
ents were similar in size but both the largest and the smallest
were readily distinguished. The X-chromosome was approxi-
mately half of the size of the smallest half-bivalent and tended
to be placed at the periphery of the metaphase plate. The afore-
mentioned size structure of the karyotype was con-
firmed 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 Crypto-
neossa, 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 access-
ory glands are similar in shape to seminal vesicles with a
slightly elongated form. Each testis consists of a single fol-
llicle. 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 speri-
ogenesis (Fig. 5). Within the meiotic zone, spermatocysts are
arranged without any apparent alignment. An absence of
alignment is also characteristic of the zone of speriogenesis,
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 pre-
sented here) from 51 psylliid genera have been studied
(Maryʻanska-Nadachowska et al. 1992; Kuznetsova et al.
1997a; Matcharashvili & Kuznetsova 1997; Maryʻanska-
Nadachowska et al. 2001a). These figures include all fami-
lies except Phacocteronidae. 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 chromo-
some 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 psylliid possessed this karyo-
type (Kuznetsova et al. 1995, 1997a; Matcharashvili &
Kuznetsova 1997) and it could be argued that all other karyo-
types in the Psylloidea could have evolved as derived charac-
ters 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 *Triozza* Förster (Triozidae). In *Triozza ilicina* (de Stefani Perez) and *T. remotae* 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 *Triozza* 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 & Hallka 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 neo-chromosomes 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 Rhinocoleinae (Aphalaridae), *Agonosca cisti* (Puton), *A. targinii* (Lichtenstein) and *Lisonia 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 *Pachypylla venusta* (Osten-Sacken) and $2n = 21$ (20 + X) in *Pa. celldisgemma* Riley.

Karyotypes of Spondylaspidinae 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 Spondylaspidinae, 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 Spondylaspidinae, given that two genera, *Cardiaspina* and *Cryptoneossa*, had species with variable chromosome numbers (e.g. *Ca. albitectura* possess $2n = 11$ and *Ca. retator* had $2n = 9$, and *Cryptoneossa* (near triangula) possesses $2n = 9$ and *Cr. vulgaris* had $2n = 7$). All spondylaspidine 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 Spondylaspidinae (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 spondylaspidine Psylloidea.

Taylor (1990) recognised four tribes (Ctenarytainini, Euphalerini, Euphyllurini and Spondylaspidini). 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 *Blastosypylla* 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*, *Blastosypylla* and *Cryptoneossa*), of which *Anoeconeossa* constitutes the most derived condition.

Of the Spondylaspidini, 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*, *Creis* and *Spondylaspis* 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 Spondylaspidini 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 Spondylaspidini.
Male reproductive system and arrangement of spermatocysts in follicles

The male internal reproductive system is similar in all psyllid species studied (Głowacka 1983; Głowacka & Maryńska-Nadachowska 1993). We confirm this for the Spondylaspidae. 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 (Głowacka 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 (Głowacka et al. 1995). In some species, however, spermatocysts show a random distribution, their outline often difficult to distinguish (Głowacka & Maryńska-Nadachowska 1998; present study). This arrangement appears to be characteristic of all species with short oval follicles such as Ct. eucalypti (Głowacka et al. 1995) and Mycopsylla fici (Tryon) (Głowacka & Maryńska-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 (Głowacka 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, Agonosccena Enderlein, Listromia Loginova, Megagonosccena and Taiuarys Bréthes); in Carsidarinae (Carsidariinae) with all three genera studied (Tenaphalara Kuwayama, Mesohomotoma Kuwayama, and Paracarsidara Heslop-Harrison); and in Pachysyliniae (the sister group of Spondylaspidae (Burckhardt 1991)) with both genera studied (Pachysylla Riley and Tetragonocephala Crawford).

The present study suggests that one follicle per testis is a plesiomorphic character within the Spondylaspidae, occurring in all 17 species referred to all 11 spondylaspide genera (Table 2). But one exception to this configuration in the Spondylaspidae is where two follicles per testis have been reported for Spondylapis sp. (Głowacka et al. 1995). This may indicate that one follicle per testis may constitute the ancestral character for Spondylaspidae and Pachysyliniae and that two follicles represents the derived condition, having apparently evolved once in the Spondylaspidae. 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 Spondylaspidae and Aphalaridae with the Psyllidae (Burckhardt 1987), the Euphyllurini and Ctenarytainini were transferred to the Spondylaspidae. Consistent with the classification by Taylor (1990) is that two species of Ctenarytainini, Ct. eucalypti (Głowacka et al. 1995) and Ctenarytaina sp. (present study) (referred to the Spondylaspidae) both possess one follicle per testis, whereas two species of Euphyllura Förster (E. olivina Costa and E. phillyreae Förster) display two follicles per testis (Głowacka 1983). A similarity in the testis structure of Ctenarytaina and the genera Anoeconoeossa, 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 Spondylaspidae and Pachysyliniae or in each of these groups independently. Species in the genus Euphyllura and one of the two species of Spondylapis studied (Głowacka 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 spondylaspidines 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 (Maryńska-Nadachowska et al. 1992; Kuznetsova et al. 1997a,b). For the Spondylaspidae (sensu White & Hodkinson 1985) (Psyllidae: Spondylaspidae (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 Spondylaspidae as a compact, advanced and specialised taxon that has evolved very intensive rearrangements of its genetic structure in its evolution.

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REFERENCES


Taylor KL. 1990. The tribe Ctenarytainini (Homoptera: Psylloidea): A key to known Australian genera, with new species and two new genera. Invertebrate Taxonomy 4, 95–121.


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