

## A PRELIMINARY MOLECULAR ANALYSIS OF PHYLOGENETIC RELATIONSHIPS OF AUSTRALASIAN WOLF SPIDER GENERA (ARANEAE, LYCOSIDAE)

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**ABSTRACT.** A data-set from the mitochondrial 12S rRNA gene subunit of 11 Australasian lycosid species (six New Zealand species and five Australian species) was generated. Three North American lycosid species, one European species and one New Zealand pisaurid (outgroup) were also sequenced. The sequence data for the 16 species were combined with the published sequences of 12 European lycosids, two Asian lycosids and one Asian pisaurid and were analyzed using parsimony and maximum likelihood analyses. The resulting phylogenetic trees reveal that Australasian species largely form clades distinct from Palearctic and Holarctic species providing further evidence against the placement of Australasian species in Northern Hemisphere genera. New Zealand wolf spiders appear to be related to a subset of Australian genera whereas the other Australian lycosid genera are related to Asian/Holarctic faunas. Gene sequences in the 12S region were useful when examining relationships between closely related genera, but were not as informative for deeper generic relationships.

**Keywords:** Lycosidae, New Zealand, Australia, lycosid genera, lycosid subfamilies

The monophyly of the Lycosidae is well supported (e.g. Dondale 1986; Griswold 1993), but at the subfamily level there is some disagreement (Dondale 1986; Zyuzin 1993; Dippenaar-Schoeman & Jocqué 1997) and lycosid genera, many of which are paraphyletic and polyphyletic, are in disarray. Although European lycosid generic placements are well established (e.g. Heimer & Nentwig 1991) and some Nearctic and African genera have been recently revised (e.g. Alderweireldt 1991, 1999; Dondale & Redner 1978, 1979, 1983a, 1983b; Russell-Smith 1982), a large number of the 2245 lycosid species (Platnick 2001) would seem to be misplaced. For example, a revision of the New Zealand lycosid fauna (Vink 2002) found that all but one described species were incorrectly placed in mostly Northern Hemisphere genera. Some of the confusion can be attributed to Roewer (1951, 1955, 1959, 1960) who described 65 lycosid genera of which only 31 are currently recognized (Platnick 2001); 12 of these are monotypic and many others contain only two species. Roewer's generic descriptions were short and based on highly variable, non-genitalic characters. Brignoli (1983) stated "it is apparent that most recent students of this

group give little value to most of the genera described by Roewer in 1954 [1955] and 1960; still it is necessary to list them as no acceptable new 'system' has been yet proposed". However, Roewer cannot be held entirely responsible for the state of lycosid genera. Many of the generic problems are due to the morphological conservatism of the Lycosidae and the consequential lack of useful characters to define and separate genera.

In New Zealand and Australia, many early workers placed lycosid species into genera with which they were familiar in their native Europe (e.g. Koch 1877). In particular, *Lycosa* Latreille 1804, which is now considered to be a Mediterranean genus (Zyuzin & Logunov 2000), has been a convenient genus in which to place many new species or as a temporary home when genera need revising (e.g. McKay 1975). Many of the large, burrow-dwelling Australian species have been placed in *Lycosa* (e.g. *Lycosa godeffroyi* L. Koch 1865) but do not fit the genus as defined by Zyuzin & Logunov (2000). Rather, they have a genitalic morphology similar to *Geolycosa* Montgomery 1904 (sensu Dondale & Redner 1990).

Lycosids are among the numerically dominant arthropod predators found in open habi-

tats in Australasia (e.g. Forster 1975; Humphreys 1976; Churchill 1993; Sivasubramaniam et al. 1997; Hodge & Vink 2000; Framenau et al. 2002) and recent taxonomic work (Framenau 2002; Framenau & Vink 2001; Vink 2001, 2002) has addressed the generic placement of some Australasian species. New Zealand's fauna, comprising 27 species, has been revised (Vink 2002) with most species (20) in *Anoteropsis* L. Koch 1878. The Australasian genera *Allotrochosina* Roewer 1960 (two species), *Artoria* Thorell 1877 (17 species), *Notocosa* Vink 2002 (one species) and *Venatrix* Roewer 1960 (22 species) have been recently revised or reviewed (Framenau 2002; Framenau & Vink 2001; Vink 2001, 2002). There are also 12 Australian species that form "a natural grouping" and were placed in *Trochosa* C.L. Koch 1848 (McKay 1979) but none of these species fit the genus as defined by Dondale & Redner (1990). Australia has 141 described lycosid species and at least another 100 undescribed species (V.W. Framenau pers. comm.; CJV pers. obs.). The majority of Australian species appear to belong in *Artoria* and a *Geolycosa*-like genus (V.W. Framenau pers. comm.; CJV pers. obs.). Species in *Venatrix* and the *Geolycosa*-like genus have a pedipalpal configuration that places them in the Lycosinae Simon 1898 (Framenau & Vink 2001; CJV pers. obs.). Vink (2001) placed *Allotrochosina* in Venoniinae Lehtinen & Hippa 1979 (sensu Dondale 1986) and while the simple pedipalps of *Anoteropsis*, *Artoria*, *Notocosa* and the Australian species currently in *Trochosa* do not fit any of the current subfamily definitions (Framenau 2002; Vink 2002; CJV pers. obs.) they are perhaps closest to Venoniinae (sensu Dondale 1986). The phylogenetic position of Australasian genera within the Lycosidae is unknown.

Because lycosids are morphologically conservative it is unlikely that sufficient numbers of morphological characters could be found to infer phylogenetic relationships of Australasian genera to their counterparts in the rest of the world. Sequence data from a portion of the mitochondrial 12S rRNA gene of the small ribosomal subunit have yielded large data sets for phylogenetic analysis of spiders (e.g. Gillespie et al. 1994). Recently, 12S rRNA sequence data have been used to infer relationships among European lycosids (Zehethofer & Sturmbauer 1998; Vink & Mitchell 2002) and

the relationship of Asian lycosids to other Lycosoidea (Fang et al. 2000). Zehethofer & Sturmbauer (1998) found that 12S rRNA was especially suitable for resolving relationships higher than the species level.

This preliminary study aimed to examine the relationship of exemplars of the major Australasian genera to exemplars of genera found elsewhere in the world using phylogenetic analyses of 12S rDNA sequence data.

## METHODS

Generic placement of species was based on the latest catalog of Platnick (2001) and recent taxonomic revisions (Framenau 2002; Framenau & Vink 2001; Vink 2001, 2002). Species sequenced, sex, and collection details (locality, date and collectors) are shown in Table 1. All specimens are stored in 95% ethanol and refrigerated in the Ecology & Entomology Group, Lincoln University. Selected Australasian species represented the major species groups of Australia and New Zealand (Framenau 2002; Framenau & Vink 2001; Vink 2001, 2002; CJV pers. obs.). The North American species *Geolycosa rogersi* Wallace 1942, *Varacosa avara* (Keyserling 1877) and *Allocosa georgicola* (Walckenaer 1837) were sequenced and included in the analysis because of the similarity of their male pedipalpal morphology to *Lycosa godeffroyi*. It should be noted that *Allocosa georgicola* does not fit the genus *Allocosa* Banks 1900 as defined by Dondale & Redner (1983b).

**DNA extraction, amplification and sequencing.**—Specimens were washed in sterile deionized, distilled water before DNA extraction. Total genomic DNA was extracted by homogenizing 1–2 legs from single individuals (Table 1) using a proteinase-K digestion and high salt precipitation method (White et al. 1990). Mitochondrial 12S regions were amplified using the following two primer combinations:

- 1) 12St-L (5'-GGTGGCATTATTTATTTAT-TAGAGG-3') (Croom et al. 1991) plus 12Sbi-H (5'-AAGAGCGACGGGCGATGTGT-3') (Simon et al. 1990), or
- 2) 12SR-N-14594 (5'-AAACTAGGATTAG-ATACCC-3') plus 12SR-J-14199 (5'-TACTATGTTACGACTTAT-3') (Kam-bhampati & Smith 1995) (Fig. 1).

Each 25  $\mu$ l reaction consisted of 1 $\times$  *Taq*

Table 1.—Specimens sequenced showing species, sex, collection localities, collectors and dates collected, primers used and GenBank accession numbers.

Species	Sex	Collection details	Primers used	GeneBank accession no.
<i>Allocosa georgicola</i> (Walckenaer 1837)	female	USA, near Oxford (34°13'N, 89°19'W), 12.x.1999, L. Schaffer	12SR-J + 12SR-N	AF380499
<i>Alopecosa barbipes</i> (Sundevall 1833)	male	England, Redgrave & Lopham Fen (52°23'N, 01°00'E), 6.x.1999, C.J. Vink & M.A. Hudson	12St-L + 12Sbi	AY028420
<i>Allotrochosina schauinslandi</i> (Simon 1899)	female	New Zealand, Prices Valley (43°48'S, 172°41'E), 12.vi.1999, C.J. Vink & J.W. Griffiths	12St-L + 12Sbi	AF380502
<i>Anoteropsis adumbrata</i> (Urquhart 1887)	female	New Zealand, Titan Rocks (45°32'S, 169°00'E), 9.xii.1998, G. Hall, B. Brown & E. Edwards	12St-L + 12Sbi	AF380491
<i>Anoteropsis lacustris</i> Vink 2002	male	New Zealand, Arthur's Pass (42°56'S, 171°34'E), 9.iv.1999, C.J. Vink & M.A. Hudson	12St-L + 12Sbi	AF380489
<i>Anoteropsis senica</i> (L. Koch 1887)	male	New Zealand, Franz Josef Glacier (43°25'S, 170°10'E), iv.1999, C.J. Vink & M.A. Hudson	12SR-J + 12SR-N	AF380490
<i>Arctoria flavimanus</i> Simon 1909	male	Australia, Crowea (34°28'S, 116°10'E), 6.v.1999, C.J. Vink	12SR-J + 12SR-N	AF380492
<i>Dolomedes minor</i> L. Koch 1876	female	New Zealand, Lake Ellesmere (43°43'S, 172°30'E), 20.xi.1999, R.M. Emberson	12SR-J + 12SR-N	AF380503
<i>Geolycosa rogersi</i> Wallace 1942	female	USA, Avent Park 34°13'N, 89°18'W), 1.iv.2000, G. Stratton, P. Miller & B. Suter	12SR-J + 12SR-N	AF380498
<i>Lycosa godeffroyi</i> L. Koch 1865	female	Australia, Bellerive (42°52'S, 147°22'E), 11.v.1999, C.J. Vink & J. Cossum	12SR-J + 12SR-N	AF380497
<i>Notocosa bellicosa</i> (Goyen 1887)	male	New Zealand, Temuka (44°14'S, 171°17'E), iii.1999, M. Ross	12SR-J + 12SR-N	AF380493
<i>Trochosa oraria</i> (L. Koch 1876)	female	Australia, Lauderdale (42°55'S, 147°29'E), 11.v.1999, C.J. Vink & J. Cossum	12St-L + 12Sbi	AF380501
<i>Varacosa avara</i> (Keyserling 1877)	male	USA, Sardis Reservoir (34°15'N, 89°28'W), 14.ix.1999, G. Stratton & W. Calvert	12SR-J + 12SR-N	AF380500
<i>Venatrix goyderi</i> (Hickman 1944)	female	New Zealand, near Matarau (35°38'S, 174°11'E), 15.ii.1999, C.J. Vink	12St-L + 12Sbi	AF380496
<i>Venatrix lapidosa</i> (McKay 1974)	male	Australia, Avon River (37°48'S, 146°57'E), iii.1999, V.W. Framenau	12SR-J + 12SR-N	AF380495
<i>Venatrix pictiventris</i> (L. Koch 1877)	male	Australia, Queens Domain (42°52'S, 147°19'E), 9.v.1999, C.J. Vink	12St-L + 12Sbi	AF380494

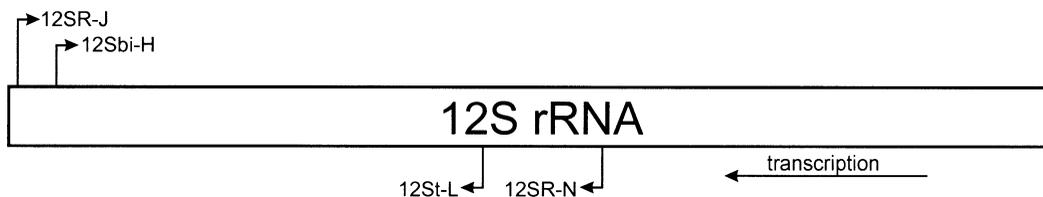


Figure 1.—Gene region coding for 12S rRNA showing areas sequenced by primers and direction of transcription.

buffer, 0.25 mM dNTPs, 2 mM MgCl<sub>2</sub>, 0.4 μM of each primer, 1.25 units *Taq* DNA Polymerase (Roche) and 1 μl of genomic DNA [which was diluted 1:20 in TE (10 mM Tris, 1 mM EDTA, pH 8.0) and used as a template for the amplification of double-stranded DNA (dsDNA)]. Amplification was performed in a GeneAmp® PCR System 2400 (Perkin-Elmer) thermocycler and the following temperature profile was used: 4 min. at 94 °C; 40 cycles of 20 s at 94 °C, 30 s at 50 °C, 40 s at 72 °C; 2 min. at 72 °C. Excess primers and salts were removed from the resulting dsDNA by precipitation with 100% isopropanol in the presence of 2.5M NH<sub>4</sub>Ac, followed by a 70% ethanol wash. Purified PCR fragments were sequenced using ABI PRISM® BigDye™ termination mix version 1 (Perkin-Elmer) and separated on an ABI PRISM® 373 automatic sequencer. The sense and antisense strands were sequenced for all species except *Venatrix pictiventris* L. Koch 1877 and *Anoteropsis la-*

*custris* Vink 2002, which were successful only one way. Sequence data were deposited in GenBank (Benson et al. 2000) (see Table 1 for accession numbers).

**Data analysis.**—Sequences were aligned to 15 previously published sequences (Zehethofer & Sturmbauer 1998; Fang et al. 2000) (Table 2) using Clustal W 1.7 (Thompson et al. 1994), then confirmed by eye. Insertion/deletion events were inferred where necessary based on the secondary structure of 12S rRNA proposed by Hickson et al. (1996). Although Hickson et al. (1996) used the 12S sequence of *Tetragnatha mandibulata* Walckenaer 1842 when constructing their template, helix 42 did not seem to be present in the lycosid or pisaurid sequences. In order to match the data obtained by Zehethofer & Sturmbauer (1998) sequence data that began five bases downstream from where the 12St-L primer annealed to seven bases upstream from where the 12Sbi-H primer annealed were included in

Table 2.—Other published sequences used in analyses showing species, reference and Genebank accession numbers.

Species	Reference	GenBank accession no.
<i>Alopecosa accentuata</i> (Latreille 1817)	Zehethofer & Sturmbauer (1998)	AJ008022
<i>Alopecosa pulverulenta</i> (Clerck 1757)	Zehethofer & Sturmbauer (1998)	AJ008025
<i>Arctosa leopardus</i> (Sundevall 1833)	Zehethofer & Sturmbauer (1998)	AJ008032
<i>Dolomedes raptor</i> Bösenberg & Strand 1906	Fang et al. (2000)	AF145031
<i>Lycosa coelestis</i> L. Koch 1878	Fang et al. (2000)	AF145030
<i>Pardosa agrestis</i> (Westring 1861)	Zehethofer & Sturmbauer (1998)	AJ008033
<i>Pardosa hortensis</i> (Thorell 1872)	Zehethofer & Sturmbauer (1998)	AJ008007
<i>Pardosa palustris</i> (Linnaeus 1758)	Zehethofer & Sturmbauer (1998)	AJ008011
<i>Pardosa takahashii</i> (Saito 1936)	Fang et al. (200)	AF145032
<i>Pirata hygrophilus</i> Thorell 1872	Zehethofer & Sturmbauer (1998)	AJ008015
<i>Pirata knorri</i> (Scopoli 1763)	Zehethofer & Sturmbauer (1998)	AJ008019
<i>Trochosa terricola</i> Thorell 1856	Zehethofer & Sturmbauer (1998)	AJ008017
<i>Trochosa spinipalpis</i> (F.O.P.-Cambridge 1895)	Zehethofer & Sturmbauer (1998)	AJ008016
<i>Xerolycosa miniata</i> (C.L. Koch 1834)	Zehethofer & Sturmbauer (1998)	AJ008021
<i>Xerolycosa nemoralis</i> (Westring 1861)	Zehethofer & Sturmbauer (1998)	AJ008020

the analyses. The analyses were conducted using PAUP\* 4.0b4a (Swofford 2000).

Data were analyzed as unordered characters, first using parsimony and the heuristic search (1000 replicates) option in PAUP\*. All characters were equally weighted, and zero length branches were collapsed to polytomies. Bootstrap values (Felsenstein 1985) were calculated from 1000 replicate parsimony analyses using the heuristic search option in PAUP\*. Modeltest version 3.06 (Posada & Crandall 1998) was used to select the maximum likelihood parameters, GTR+ $\Gamma$ +I. The general time reversible model (Yang 1994) was used to estimate the maximum likelihood tree and branches were collapsed (creating polytomies) if the branch length was less than or equal to  $1e-08$ . The maximum likelihood analysis included 20 taxa. Taxa were pruned if they were part of a well-supported node (bootstrap value >75%) in the parsimony tree leaving one representative of each taxon. Bootstrap values were calculated from 100 replicate likelihood analyses using the heuristic search option in PAUP\*.

## RESULTS

The primer combination 12St-L plus 12Sbi-H produced a single amplification product for seven species (see Table 1), but two or more bands were amplified for all other taxa. The primer pair 12SR-J-14199 plus 12SR-N-14594 was used to amplify product for sequencing for the taxa that did not produce a single amplification product using the 12St-L plus 12Sbi-H combination (see Table 1). The 12St-L primer site varied considerably in the nine taxa for which the primer pair 12SR-J-14199 plus 12SR-N-14594 was used, which may explain why the primer combination 12St-L plus 12Sbi-H did not work for all taxa. The primer 12St-L was designed as a *Tetragnatha*-specific primer (Croom et al. 1991) so it is not surprising that this site varies in lycosids. There was little variation evident in the 12Sbi-H site even though this primer was designed as specific to insects (Simon et al. 1990). The nucleotide composition was A + T-rich (44.2% A, 10.0% C, 9.8% G, 36.0% T), which is typical for arthropods (Simon et al. 1994).

Parsimony analysis yielded 2 equally parsimonious trees (Fig. 2), 482 steps long, with a consistency index, excluding uninformative

characters, of 0.415 and retention index of 0.577. Of the 330 characters included in the analysis, 172 were variable with 113 of them parsimony informative. Maximum likelihood analysis resulted in six trees with scores of 2092.1969 (Fig. 3). The six trees had the same topology because the branches were collapsed (creating polytomies) if the branch length was less than or equal to  $1e-08$ . The topology of the maximum likelihood trees (Fig. 3) and the parsimony trees (Fig. 2) differed mainly in the lower branches, which had less than 50% bootstrap support.

## DISCUSSION

Molecular analysis confirms that most of the New Zealand or Australian lycosids included in the analysis do not belong in the Northern Hemisphere genera where they have been or are currently placed. This study confirms that *Trochosa oraria* L. Koch 1876 does not belong in the genus *Trochosa* (sensu Dondale & Redner 1990) and the two Holarctic exemplars of *Trochosa* are monophyletic, which is supported by high bootstrap values (Fig. 2). There is support for the monophyly of *Pardosa* C.L. Koch 1847 as the four exemplars form a monophyletic clade that is supported by a high bootstrap value (Fig. 3). Zehethofer & Sturmbauer (1998) also had strong support for the monophyly of the 14 exemplars of *Pardosa* that they included in their analysis. The three exemplars of *Alopecosia* Simon 1885 included in this study form a strongly supported monophyletic clade, as did the six exemplars included in the analysis of Zehethofer & Sturmbauer (1998). The exemplars of *Xerolycosa* Dahl 1908 and *Pirata* Sundevall 1833 both have good support for their monophyly. The molecular evidence suggests that *Allocosa georgicola* belongs in a *Geolycosa*-like genus, however, there is poor bootstrap support and no *Allocosa* species (sensu Dondale & Redner 1983b) were included in this analysis. *Lycosa coelestis* L. Koch 1878 does not fit the genus *Lycosa* as defined by Zyuzin & Logunov (2000) and comes out as sister to *Varacosa avara* in both analyses with reasonable bootstrap support. However, Dondale & Redner (1990) stated that *Varacosa* Chamberlin & Ivie 1942 is restricted to North America. Both trees (Figs. 2, 3) support the monophyly of the clade containing spiders with *Geolycosa*-like pedipalps

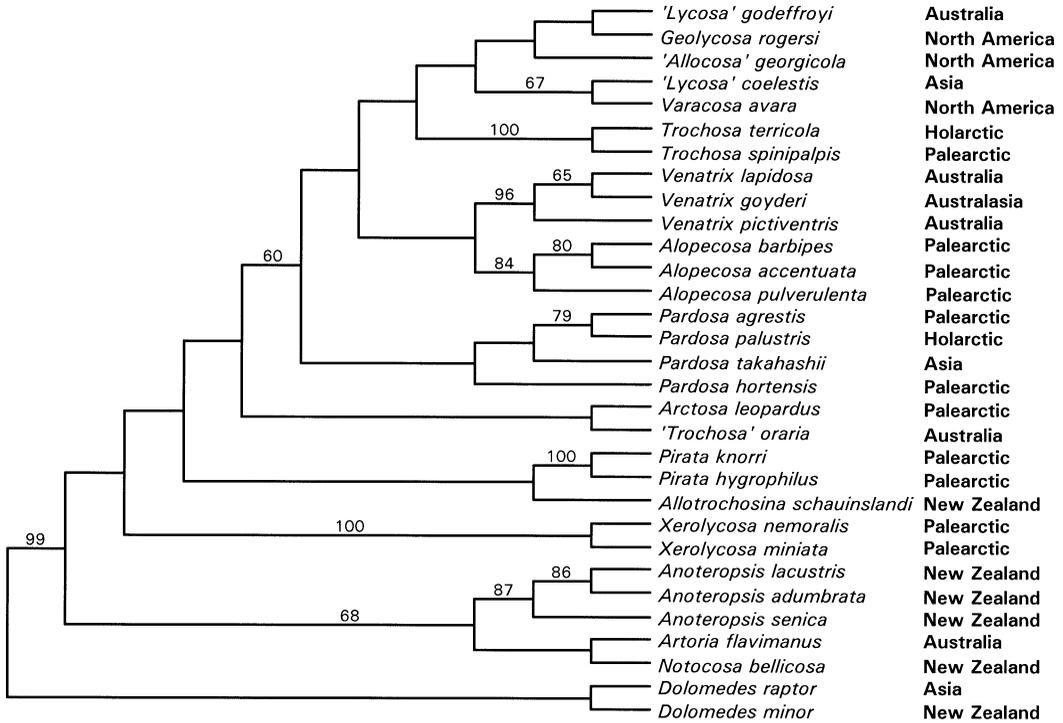


Figure 2.—One of two most parsimonious trees. The other tree differed by switching the positions of *Lycosa godeffroyi* and *Allocosa georgicola*. Bootstrap values above 50% are indicated above branches. Species distributions based on Platnick (2001) are shown on the right. Species that do not fit current generic definitions have the generic name in inverted commas.

(*L. godeffroyi*, *G. rogersi*, *A. georgicola*, *L. coelestis* and *V. avara*) but there is low (< 50%) bootstrap support for this clade. The Mediterranean genus *Lycosa* (sensu Zyuzin & Logunov 2000) is unlikely to be appropriate for *L. godeffroyi* but this cannot be inferred from our analyses because we did not sequence any Mediterranean *Lycosa* species. However, both analyses have *L. godeffroyi* coming out with *Geolycosa rogersi*, which is a true *Geolycosa*. The strongly supported, monophyletic clade of three *Venatrix* exemplars supports the monophyly of *Venatrix*. In both analyses (Figs. 2, 3) *Venatrix* was sister to *Alopecosa* and it has been noted that they share a similar pedipalpal structure (Framenau & Vink 2001). The clade containing the three *Anoteropsis* exemplars is monophyletic, which concurs with Vink (2002). *Anoteropsis* and *Notocosa* appear to be restricted to New Zealand (Vink 2002) and *Artoria* are most diverse in Australia but are also found in New Zealand, Papua New Guinea and the Philippines (Framenau 2002; Vink 2002). The

monophyly of the clade containing exemplars from *Anoteropsis*, *Artoria* and *Notocosa* is supported in both analyses and all five species share a similar pedipalp configuration (Figs. 4–8) that includes a partially divided tegulum and similarities in the position and shape of the median apophysis (Vink 2002). The relationship of *Notocosa bellicosa* (Goyen 1887) to the other four species in the clade differs between the analyses. The parsimony analysis puts *N. bellicosa* as sister to *Artoria flavimanus* Simon 1909, whereas the bootstrap support (61%) within the parsimony trees and maximum likelihood analysis have *N. bellicosa* as sister to a clade containing the other four species. This clade does not fit current subfamily definitions and, once the genera are revised, may be placed in its own subfamily.

When *Trochosa oraria* is not included in *Trochosa*, the subfamilies Pardosinae Simon 1898 and Lycosinae Simon 1898 as defined by Dondale (1986) are supported, except for *Arctosa* C.L. Koch 1847, which falls outside the Lycosinae in this analysis. Dondale (1986)

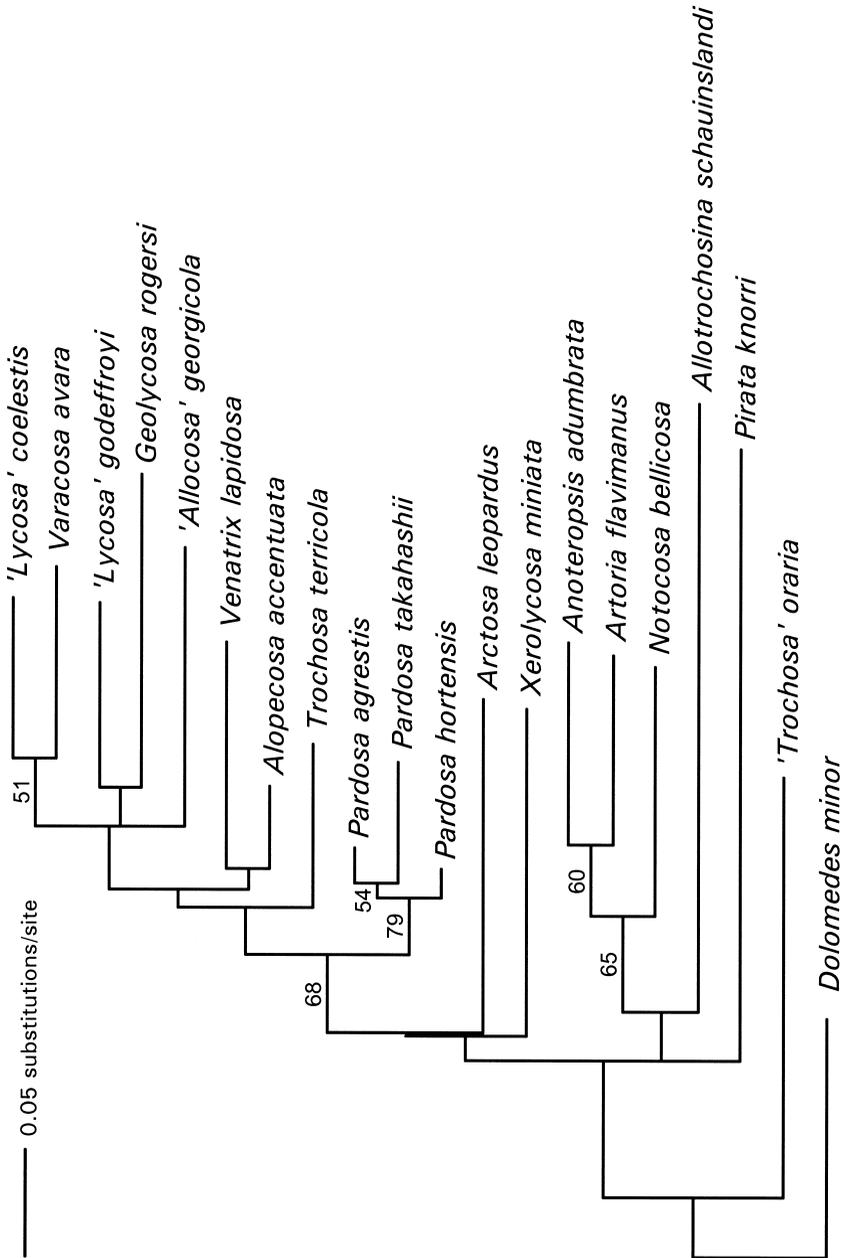
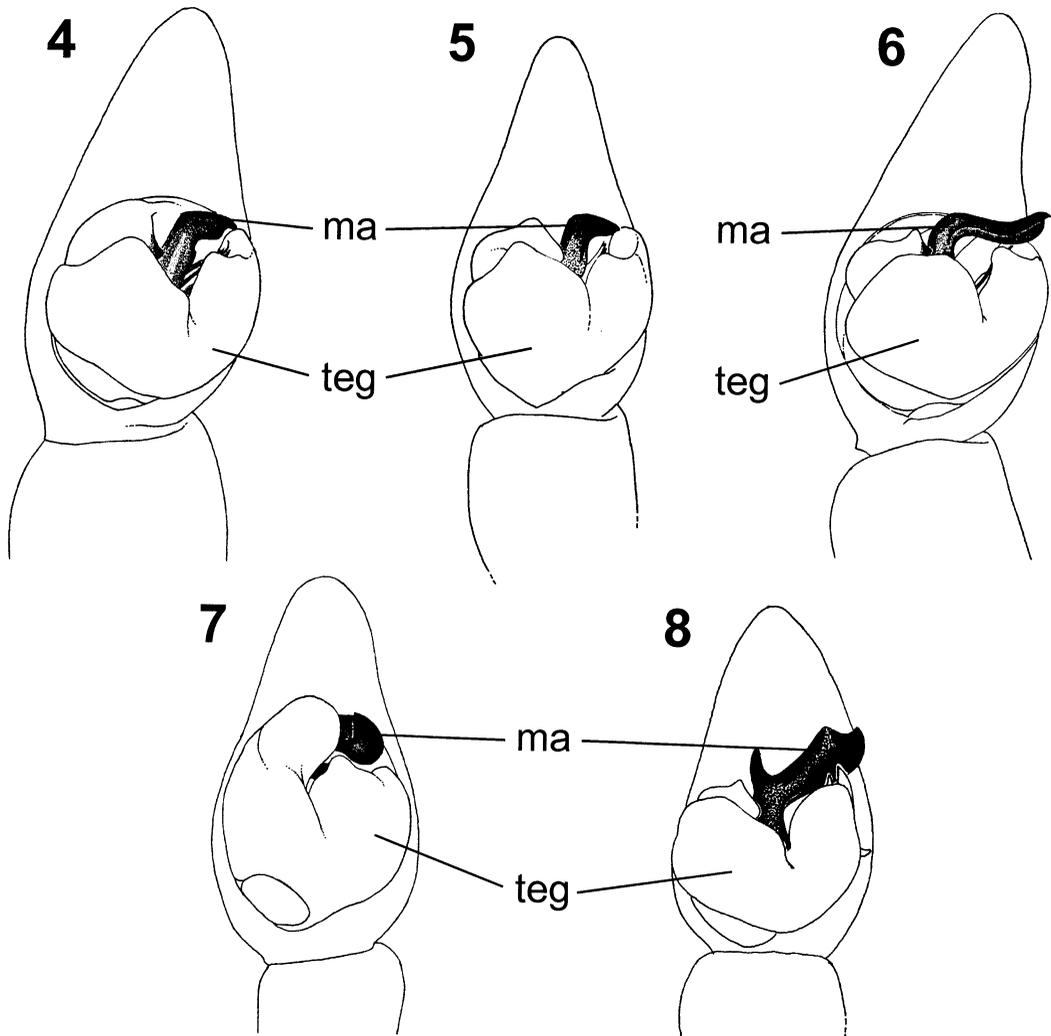


Figure 3.—Strict consensus of the six maximum likelihood trees. Bootstrap values above 50% are indicated above branches. Branch lengths are proportional to nucleotide substitutions. Species that do not fit current generic definitions have the generic name in inverted commas.



Figures 4–8.—Palps of (4) *Anoteropsis adumbrata*, (5) *Anoteropsis lacustris*, (6) *Anoteropsis senica*, (7) *Notocosa bellicosa* and (8) *Artoria flavimanus* showing partially divided tegulum (teg) and similarities in position and shape of median apophysis (ma).

suggested that the Lycosinae be divided into the “*Trochosa* group” and the “*Lycosa* group” but they are paraphyletic in our analyses. The placement of *Allotrochosina* in the subfamily Venoniinae (which also includes *Pirata* Sundevall 1833) by Vink (2001) is supported by the parsimony tree (Fig. 2) but not by the maximum likelihood tree (Fig. 3). It is worth noting that there is little bootstrap support for the lower branches of either tree. Further sequencing of several other genera may resolve these subfamily relationships.

While the pattern of distribution fits with a Gondwanan scenario a more detailed study of

genetic divergence may reveal a better approximation of the time the faunas have been separated. Preliminary analyses presented here (Figs. 2, 3) imply that Australasia had an ancestral fauna and was subsequently invaded by lycosine species, possibly via Asia through northern Australia. When New Zealand split away from Australia about 80 million years ago (Stevens et al. 1988), it is likely it retained an ancestral lycosid fauna. Only two lycosine species (*Venatrix goyderi* (Hickman 1944) and *Geolycosa tongatabuensis* (Strand 1911)) are found in New Zealand and it is likely that they have subsequently ballooned across to

New Zealand; both species are widely distributed across Australia and the South Pacific respectively but, in New Zealand, they are limited to the warmer north of the North Island.

The phylogenies presented here are somewhat preliminary, as some genera found in Australia are not represented (e.g. *Anomalosa* Roewer 1960, *Venonia* Thorell 1894, *Zoica* Simon 1898). Further resolution of subfamily relationships could also be facilitated by the inclusion of exemplars from Allocosinae Dondale 1986, Sosippinae Dondale 1986, Tricassininae Alderweireldt & Jocqué 1993, and Wadicossinae Zyuzin, 1985. The inclusion of at least one exemplar from *Lycosa* (sensu Zyuzin & Logunov 2000) may help to confirm the relationship of that genus to other lycosine genera.

Results presented here suggest that 12S DNA sequence data are useful for inferring phylogenies of closely related genera. However, these data appear to be too conservative for adequate resolution at the species level (Vink & Mitchell 2002) and too fast for deeper relationships, inferred from bootstrap support of less than 50% shown for the lower branches of the parsimony tree (Fig. 2). Deeper relationships in the Lycosidae may be better resolved by the use of an even more slowly evolving gene region, such as 28S rDNA, which has been used to infer spider phylogeny at the family level (Hausdorf 1999).

In summary, we conclude that many current generic placements of Australasian species are incorrect; the New Zealand fauna is related to a subset of the Australian fauna and parts of the Australian fauna are related to the Asian/Holarctic fauna, suggesting a subsequent invasion. Current subfamilies were found to be largely monophyletic but further work is required to fully resolve subfamily relationships.

#### ACKNOWLEDGMENTS

We thank the following people for help with the collection of fresh specimens: Marie Hudson, Jeff Cossum (Tasmanian Museum & Art Gallery), Volker Framenau (University of Melbourne), Grace Hall (Landcare Research), Rowan Emberson (Lincoln University) and Philip Howe (South Canterbury Museum). Thanks to Gail Stratton (University of Mississippi) for collecting and sending fresh specimens from the US. We are indebted to Dianne

Gleeson (Landcare Research) and Martyn Kennedy (University of Glasgow) for assisting with maximum likelihood analyses. Volker Framenau, Phil Sirvid and Eric Scott provided helpful comments on the manuscript. This research was made possible by funding from Landcare Research, the Miss E.L. Hellaby Indigenous Grasslands Research Trust and the Soil, Plant and Ecological Sciences Division, Lincoln University.

#### LITERATURE CITED

- Alderweireldt, M. 1991. A revision of the African representatives of the wolf spider genus *Evipa* Simon, 1882 (Araneae, Lycosidae) with notes on allied species and genera. *Journal of Natural History* 25:359–381.
- Alderweireldt, M. 1999. A revision of Central African *Trabea* (Araneae, Lycosidae) with the description of two new species from Malawi and a redescription of *T. purcelli*. *Journal of Arachnology* 27:449–457.
- Benson, D.A., I. Karsch-Mizrachi, D.J. Lipman, J. Ostell, B.A. Rapp & D.L. Wheeler. 2000. GenBank. *Nucleic Acids Research* 28:15–18.
- Brignoli, P.M. 1983. A Catalogue of the Araneae Described Between 1940 and 1981. Manchester University Press, Manchester.
- Churchill, T.B. 1993. Effects of sampling method on composition of a Tasmanian coastal heathland spider assemblage. *Memoirs of the Queensland Museum* 33:475–481.
- Croom, H.B., R.G. Gillespie & S.R. Palumbi. 1991. Mitochondrial DNA sequences coding for a portion of the RNA of the small ribosomal subunits of *Tetragnatha mandibulata* and *Tetragnatha hawaiiensis* (Araneae, Tetragnathidae). *Journal of Arachnology* 19:210–214.
- Dippenaar-Schoeman, A.S. & R. Jocqué. 1997. African Spiders: An Identification Manual. Plant Protection Research Institute, Pretoria.
- Dondale, C.D. 1986. The subfamilies of wolf spiders (Araneae: Lycosidae). *Actas X Congreso Internacional de Aracnología, Jaca, España* 1:327–332.
- Dondale, C.D. & J.H. Redner. 1978. Revision of the Nearctic wolf spider genus *Schizocosa* (Araneida: Lycosidae). *Canadian Entomologist* 110:143–181.
- Dondale, C.D. & J.H. Redner. 1979. Revision of the wolf spider genus *Alopecosa* Simon in North America (Araneae: Lycosidae). *Canadian Entomologist* 111:1033–1055.
- Dondale, C.D. & J.H. Redner. 1983a. Revision of the wolf spiders of the genus *Arctosa* C.L. Koch in North and Central America (Araneae: Lycosidae). *Journal of Arachnology* 11:1–30.
- Dondale, C.D. & J.H. Redner. 1983b. The wolf spi-

- der genus *Allocosa* in North and Central America (Araneae: Lycosidae). Canadian Entomologist 115:933–964.
- Dondale, C.D. & J.H. Redner. 1990. The Wolf Spiders, Nurseryweb Spiders, and Lynx Spiders of Canada and Alaska. Araneae: Lycosidae, Pisauridae, and Oxyopidae. Agriculture Canada, Canada.
- Fang, K., C.-C. Yang, B.-W. Lue, S.-H. Chen & K.-Y. Lue. 2000. Phylogenetic corroboration of superfamily Lycosoidea spiders (Araneae) as inferred from partial mitochondrial 12S and 16S ribosomal DNA sequences. Zoological Studies 39:107–113.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.
- Framenau, V.W. 2002. Review of the wolf spider genus *Artoria* Thorell (Araneae, Lycosidae). Invertebrate Systematics 16:in press.
- Framenau, V.W., R. Manderbach & M. Baehr. 2002. Riparian gravel banks of upland and lowland rivers in Victoria (south-east Australia): arthropod community structure and life history patterns along a longitudinal gradient. Australian Journal of Zoology 50:103–123.
- Framenau, V.W. & C.J. Vink. 2001. Revision of the wolf spider genus *Venatrix* Roewer (Araneae: Lycosidae). Invertebrate Taxonomy 15:927–970.
- Forster, R.R. 1975. The spiders and harvestmen. Pp. 493–505. In Biogeography and Ecology in New Zealand (G. Kuschel, ed.). W. Junk, The Hague.
- Gillespie, R.G., H.B. Croom & S.R. Palumbi. 1994. Multiple origins of a spider radiation in Hawaii. Proceedings of the National Academy of Sciences of the United States of America 91:2290–2294.
- Griswold, C.E. 1993. Investigations into the phylogeny of the lycosid spiders and their kin (Arachnida: Araneae: Lycosoidea). Smithsonian Contributions to Zoology 539:1–39.
- Hausdorf, B. 1999. Molecular phylogeny of araneomorph spiders. Journal of Evolutionary Biology 12:980–985.
- Heimer, S. & W. Nentwig. 1991. Spinnen Mitteleuropas: Ein Bestimmungsbuch. Verlag Paul Parey, Berlin.
- Hickson, R.E., C. Simon, A. Cooper, G.S. Spicer, J. Sullivan & D. Penny. 1996. Conserved sequence motifs, alignment, and secondary structure for the third domain of animal 12S rRNA. Molecular Biology and Evolution 13:150–169.
- Hodge, S. & C.J. Vink. 2000. An evaluation of *Lycosa hilaris* as a bioindicator of organophosphate insecticide contamination. New Zealand Plant Protection 53:226–229.
- Humphreys, W.F. 1976. The population dynamics of an Australian wolf spider, *Geolycosa godeffroyi* (L. Koch 1865) (Araneae: Lycosidae). Journal of Animal Ecology 45:59–80.
- Kambhampati, S. & P.T. Smith. 1995. PCR primers for the amplification of four insect mitochondrial gene fragments. Insect Molecular Biology 4: 233–236.
- Koch, L. 1877. Die Arachniden Australiens. Bauer and Raspe, Nürnberg.
- McKay, R.J. 1975. The wolf spiders of Australia (Araneae: Lycosidae): 5. Two new species of the *bicolor* group. Memoirs of the Queensland Museum 17:313–318.
- McKay, R.J. 1979. The wolf spiders of Australia (Araneae: Lycosidae): 13. The genus *Trochosa*. Memoirs of the Queensland Museum 19:277–298.
- Platnick, N.I. 2001. The World Spider Catalog. <http://research.amnh.org/entomology/spiders/catalog81–87>. The American Museum of Natural History.
- Posada, D. & K.A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.
- Roewer, C.F. 1951. Neue Namen einiger Araneen-Arten. Abhandlungen des Naturwissenschaftlichen Vereines zu Bremen 32:437–456.
- Roewer, C.F. 1955 (imprint date 1954). Katalog der Araneae von 1758 bis 1940. Institut Royal de Sciences Naturelles de Belgique, Bruxelles.
- Roewer, C.F. 1959 (imprint date 1958). Araneae Lycosaeformia II (Lycosidae). Exploration du Parc National de l'Upemba 55:1–518.
- Roewer, C.F. 1960 (imprint date 1959). Araneae Lycosaeformia II (Lycosidae) (Fortsetzung und Schluss). Exploration du Parc National de l'Upemba 55:519–1040.
- Russell-Smith, A. 1982. A revision of the genus *Trabaea* Simon (Araneae: Lycosidae). Zoological Journal of the Linnean Society 74:69–91.
- Simon, C., S. Pääbo, T.D. Kocher & A.C. Wilson. 1990. Evolution of the mitochondrial ribosomal RNA in insects as shown by the polymerase chain reaction. Pp. 235–244. In Molecular Evolution, U.C.L.A. Symposia on Molecular and Cellular Biology, New Series, Vol. 122 (M. Clegg & S. O'Brien, eds.). Liss, New York.
- Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu & P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87:651–701.
- Sivasubramaniam, W., S.D. Wratten & J. Klimaszewski. 1997. Species composition, abundance, and activity of predatory arthropods in carrot fields, Canterbury, New Zealand. New Zealand Journal of Zoology 24:205–212.
- Stevens, G.R., M. McGlone & B. McCulloch. 1988.

- Prehistoric New Zealand. Heinemann Reed, Auckland.
- Swofford, D.L. 2000. PAUP\*: Phylogenetic analysis using parsimony (and other methods), version 4.0b4a. Sinauer Associates, Sunderland, Massachusetts.
- Thompson, J.D., D.G. Higgins & T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673–4680.
- Vink, C.J. 2001. A revision of the genus *Allotrichosina* Roewer (Lycosidae: Araneae). *Invertebrate Taxonomy* 15:461–466.
- Vink, C.J. 2002. Lycosidae (Arachnida: Araneae). Fauna of New Zealand, in press.
- Vink, C.J. & A.D. Mitchell. 2002. 12S DNA sequence data confirms the separation of *Alopecosa barbipes* and *Alopecosa accentuata* (Araneae: Lycosidae). *Bulletin of the British Arachnological Society* 12:in press.
- White, T.J., T. Bruns, S. Lee & J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322. *In* PCR Protocols: A Guide to Methods and Applications (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds.). Academic Press, San Diego.
- Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. *Journal of Molecular Evolution* 39:306–314.
- Zehethofer, K. & C. Sturmbauer. 1998. Phylogenetic relationships of Central European wolf spiders (Araneae: Lycosidae) inferred from 12S ribosomal DNA sequences. *Molecular Phylogenetics and Evolution* 10:391–398.
- Zyuzin, A.A. 1993. Studies on the wolf spiders (Araneae: Lycosidae). I. A new genus and species from Kazakhstan, with comments on the Lycosidae. *Memoirs of the Queensland Museum* 33: 693–700.
- Zyuzin, A.A. & D.V. Logunov. 2000. New and little-known species of the Lycosidae from Azerbaijan, the Caucasus (Araneae, Lycosidae). *Bulletin of the British Arachnological Society* 11: 305–319.

*Manuscript received 1 July 2001, revised 4 February 2002.*