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# Molecular Phylogeny of East Asian Wolf Spiders (Araneae: Lycosidae) Inferred from Mitochondrial 12S Ribosomal DNA

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**ABSTRACT** This study investigates molecular phylogenetic relationships of East Asian wolf spiders (Araneae: Lycosidae), focusing on species from Korea. We analyzed a sequence data set making up a portion of the mitochondrial 12S gene from 22 Korean lycosid species, belonging to seven genera (*Alopecosa*, *Arctosa*, *Lycosa*, *Pardosa*, *Pirata*, *Trochosa*, and *Xerolycosa*) in four subfamilies (Evippinae, Lycosinae, Pardosinae, and Venoniinae). All Korean wolf spiders were well grouped within monophyletic clades corresponding to existing genera, with the exception of *Arctosa* and *Alopecosa*, which were both polyphyletic. The genus *Arctosa* formed two distantly separated clades, and the clade composed of *A. kwangreungensis* and *A. ebicha* was posited as the sister group of all the other Korean wolf spiders. We also combined our data set with 44 previously published sequences from other lycosids from various geographical regions. As with the analyses for Korean taxa, the genus *Arctosa* still contained two distantly separated clades; the clade made up of *A. kwangreungensis* and *A. ebicha* was a sister clade to all the other lycosid spiders, whereas the clade of *A. subamylacea* and *A. leopardus* was a sister clade to that of the Australasian genera (*Anoteropsis*, (*Artoria*, *Notocosa*)). *Alopecosa cinnameopilosa* remained as a sister clade to all *Pardosa* group. The results indicate that the phylogenetic placement of the two *Arctosa* species (*A. kwangreungensis* and *A. ebicha*) and *A. cinnameopilosa* warrant reconsideration in further studies at the subfamily and generic levels, respectively.

**KEY WORDS** Araneae, Lycosidae, wolf spider, molecular phylogeny, *Arctosa*

Spiders of the family Lycosidae (wolf spiders) inhabit nearly all terrestrial habitats worldwide, and they represent ecologically important arthropod predators. Lycosids are the fourth largest spider family after Salticidae, Linyphiidae, and Araneidae, and include some 2,300 species distributed over five main subfamilies (Nentwig 1987, Platnick 2006).

To date, morphology and behavior have been important characters for distinguishing lycosids from the other spider families. Lycosid spiders are easily recognized by their morphological characteristics, such as eight eyes arranged in three rows, absence of a retrolateral tibial apophysis on the male pedipalp, and specialized setae on the dorsal surface of the female's abdomen to carry young offspring (Dondale 1986, Griswold 1993). Lycosid spiders are well-known due to their unique mobile brood care behavior, involving

the carrying of a white, round egg sac on the spinnerets of females (Dondale and Redner 1990, Foelix 1996). Although morphological and behavioral characters define the family clearly, inferred relationships at the subfamily level have sometimes been incongruent (Dondale 1986, Zyuzin 1993, Vink et al. 2002). In addition, a large number of lycosid species have been misplaced at the generic level (Vink 2002, Vink et al. 2002, and references therein). Many such generic problems seem to be due to morphological conservatism and the lack of useful characters to distinguish genera (Vink et al. 2002). Thus, recent studies have depended on the mitochondrial 12S rDNA gene sequences to infer phylogenetic relationships (Zehethofer and Sturmbauer 1998, Vink et al. 2002), as has happened in some other groups such as the comb-footed spiders (Theridiidae), jumping spiders (Salticidae), and cave spiders (Nesticidae) (Gillespie et al. 1994, Hedin 1997, Hedin and Maddison 2001, Arnedo et al. 2004).

Some recent phylogenetic studies of lycosid spiders using additional molecular markers have been conducted for Australasian (Vink et al. 2002, Murphy et al. 2006) and European groups (Zehethofer and Sturmbauer 1998). In East Asia, however, the phylogenetic relationships of wolf spiders have been poorly investigated to date. We infer phylogenetic relationships of Korean wolf spiders (22 species in seven genera and

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four subfamilies) as representatives of East Asian lycosids. In addition, our data are analyzed in combination with those from previously published sequences from Asian, Australasian, central European, and North American lycosids.

### Materials and Methods

**Taxa Studied.** We used mitochondrial 12S rDNA sequences from 22 species, which covered seven genera from four subfamilies. We also constructed a data set for inferring the phylogenetic relationships of lycosid spiders more widely, including the previously published 44 sequences from Asia (two species; Fang et al. 2000), Australasia (11 species, Vink et al. 2002), central Europe (28 species, Zehethofer and Sturmbauer 1998), and North America (three species, Vink et al. 2002) plus >22 sequences of Korean taxa (Table 1). Pisauridae have been argued as one of the most closely related group to wolf spiders within the superfamily Lycosoidea (Fang et al. 2000), and therefore *D. minor* (L. Koch, 1876) and *Dolomedes raptor* (Bösenberg & Strand, 1906) were used as outgroups.

**DNA Extraction.** Legs were severed from bodies of spiders preserved in 100% ethanol and were dried on a heating block several times to remove excess ethanol. Genomic DNA was extracted using methods provided with the Genra kit. Legs were placed in a 1.5-ml microcentrifuge tube containing 300  $\mu$ l of cell lysis solution and homogenized thoroughly using a microfuge tube pestle or a pair of dissecting scissors. After adding 1.5  $\mu$ l of 20 mg/ml proteinase K solution to the tube, the cell lysate was mixed by inverting 25 times and incubated at 56°C overnight. If possible, the tube was inverted periodically during the incubation. A final incubation followed at 65°C for an hour. Samples were cooled to room temperature, and 100  $\mu$ l of protein precipitation solution was added to the lysate. After vortexing for 20 s, the lysate was put on ice for 5 min and then centrifuged at 12,000 rpm for 5 min. The supernatant containing genomic DNA was poured into a clean 1.5-ml microcentrifuge tube containing 300  $\mu$ l of 100% isopropanol. After inverting gently 25 times, the DNA mixture was kept at -20°C for 20 min, and the DNA pellet was collected by centrifugation at 12,000 rpm for 15 min. The pellet was washed with 70% ethanol and finally dissolved in 30  $\mu$ l of DNA hydration solution.

**DNA Amplification, Purification, and Sequencing.** The fragments of mitochondrial 12S gene sequences were amplified using polymerase chain reaction (PCR). Primer sequences for the amplifications of 12S gene were as follows: 12SR-N-14594 (5'-aaactaggatagataccc-3') (Kambhampati and Smith 1995) and 12Sbi-H (5'-aagagcgacggcgatgtgt-3') (Simon et al. 1990). PCR was carried out in 25- $\mu$ l volumes, by using 2.5  $\mu$ l of 1-10 dilution of genomic DNA as template. The PCR reactions included 2.5  $\mu$ l of 5  $\mu$ M each primer, 2.5  $\mu$ l of dNTPs at 10 mM, 2.5  $\mu$ l of 25 mM MgCl<sub>2</sub>, 2.5  $\mu$ l of 10X buffer, and 1 U of *Taq* Polymerase. The temperature profile for amplifying 12S gene sequences was as follows: a denaturation at 94°C for 10

min, and 35 cycles of 94°C for 30 s, 48°C for 40 s, and 72°C for 40 s followed by a final extension of 72°C for 2 min. PCR products were purified using UltraClean PCR Clean-up kit (MO BIO Laboratories, Inc., Carlsbad, CA), and  $\approx$ 50 ng of product was sequenced in 20- $\mu$ l reaction volumes by using the Big Dye Sequencing Ready Reaction kit, version 3.1 (Applied Biosystems, Foster City, CA), with the original PCR primers used as sequencing primers. Reaction products were purified by isopropanol precipitation and sequenced on a capillary DNA sequencer.

**Data Analysis.** Sequences were aligned using the ClustalX program package (Thompson et al. 1997) and manually edited using SeAl 1.0a (Rambaut 1996). This resulted in aligned sequences of 338 bp for the 22 Korean wolf spider species and two outgroup species. Alignment of the sequences revealed that *Pirata piratoides* includes an insert of 17 bp that contains only A+T content. The insert is positioned from the aligned nucleotide site 286 downstream from the forward primer (12SR-N-14594) (refer to fig. 1 in Vink et al. 2002 for the positions of the primers). With the insert excluded, the resulting data set of 321 bp was used for further analyses.

Sequences from the Korean taxa were aligned together with 44 previously published sequences of wolf spiders from Australasia, Asia, central Europe, and North America (Table 1). The 68 aligned sequences, including two outgroup species, were 383 bp. The alignment revealed that high insertion processing is present in two regions of the aligned sequences. With the exception of the Korean species *Pirata subpiraticus*, sequences of all *Pirata* spiders contained inserts of  $\approx$ 17-51 bp from the aligned sequence site 298. In contrast to the other species of genus *Trochosa*, the sequence of *T. oraria* also contained an insert of 25 bp in this region. The Korean *Alopecosa cinnameopilosa* sequence contained an unusual insert of 7 bp from the aligned sequence position 103. All inserts exhibited high A+T content. After the removal of the two regions from the alignment, the aligned sequences of 323 bp were used for molecular phylogenetic analyses of wolf spiders more widely.

**Phylogenetic Analyses.** We used both maximum likelihood (ML) and Bayesian Inference (BI) analyses. For ML analyses, we used a series of hierarchical log likelihood ratio tests, implemented using Modeltest 3.06 (Posada and Crandall 1998) to select a best-fit model for the total data set. Heuristic searches were performed using the fitted model, with stepwise addition, by using 10 random sequence additions and holding five trees at each step, and with tree bisection reconnection branch swapping. Node support was assessed using the same search procedure and with 200 bootstrap pseudoreplicates.

We performed Bayesian analysis by using MrBayes version 3.0b4 (Huelsenbeck and Ronquist 2001) and used the same procedure for both the Korean sample and the larger world sample. Data were treated as a single partition and we specified a GTR model (Nst = 6), because this is the most general model, and it is least restrictive regarding assumptions about substi-

Table 1. Specimens sequenced showing species, sex, sampling localities, distributions, references and GenBank accession numbers

Species	Sex	Sampling locality <sup>a</sup>	United States	Distribution <sup>b</sup>	Reference	Accession no.
Genus <i>Allocosa</i>						
<i>A. georgicola</i>	F	United States	United States		Vink et al. 2002	AF380499
Genus <i>Albrochoosina</i>						
<i>A. schauinslandi</i>	F	N. Zealand	New Zealand, Chatham Is.		Vink et al. 2002	AF380502
Genus <i>Alopecosa</i>						
<i>A. albostrigata</i>	F	Korea	Russia, Asia (Kazakhstan, China, Korea)		This study, Murphy et al. 2006	DQ019757
<i>A. cinnameopilosa</i>	F	Korea	Russia, Asia (China, Korea, Japan)		This study, Murphy et al. 2006	DQ019758
<i>A. licenti</i>	F	Korea	Russia, Asia (Mongolia, China, Korea)		This study, Murphy et al. 2006	DQ019759
<i>A. moriutti</i>	M	Korea	Russia, Asia (Korea, Japan)		This study, Murphy et al. 2006	DQ019760
<i>A. pulcherrulenta</i>	F	Japan	Palaearctic		This paper and Murphy et al. 2006	DQ019756
<i>A. accentuata</i>	M	C. Europe	Palaearctic		Zehethofer and Sturmhuber 1998	AJ008022
<i>A. barbipes</i>	M	England	Palaearctic		Vink et al. 2002	AY028420
<i>A. cuneata</i>	M	C. Europe	Palaearctic		Zehethofer and Sturmhuber 1998	AJ008024
<i>A. inquilina</i>	M	C. Europe	Palaearctic		Zehethofer and Sturmhuber 1998	AJ008030
<i>A. pulcherrulenta</i>	M	C. Europe	Palaearctic		Zehethofer and Sturmhuber 1998	AJ008025
<i>A. taeniatata</i>	M	C. Europe	Palaearctic		Zehethofer and Sturmhuber 1998	AJ008026
<i>A. trabalis</i>	M	C. Europe	Europe to central Asia		Zehethofer and Sturmhuber 1998	AJ008027
Genus <i>Anoteropsis</i>						
<i>A. adumbrata</i>	F	N. Zealand	New Zealand, Stewart Is.		Vink et al. 2002	AF380491
<i>A. lacustris</i>	M	N. Zealand	New Zealand		Vink et al. 2002	AF380489
<i>A. senta</i>	M	N. Zealand	New Zealand, Stewart Is.		Vink et al. 2002	AF380490
Genus <i>Arctosa</i>						
<i>A. ebicha</i>	F	Korea	Asia (China, Korea, Japan)		This study, Murphy et al. 2006	DQ019766
<i>A. kwangjuensis</i>	M	Korea	Asia (China, Korea, Japan)		This study, Murphy et al. 2006	DQ019767
<i>A. subamylaceae</i>	F	Korea	Palaearctic		This study	DQ463223
<i>A. leopardus</i>	M	C. Europe	Palaearctic		Zehethofer and Sturmhuber 1998	AJ008032
Genus <i>Arctoria</i>						
<i>A. flavimanus</i>	M	Australia	Australia		Vink et al. 2002	AF380492
Genus <i>Geolycosa</i>						
<i>G. rogersi</i>	F	USA	United States		Vink et al. 2002	AF380498
<i>G. tongatabuensis</i>			Australasia		Vink 2002	AY059959
Genus <i>Lycosa</i>						
<i>L. coelestis</i> <sup>d</sup>	F	Korea	Asia (China, Korea, Japan)		This study, Murphy et al. 2006	DQ019785
<i>L. suzukii</i>	F	Korea	Russia, Asia (China, Korea, Japan)		This study, Murphy et al. 2006	DQ019786
<i>L. coelestis</i>	F	Australia	Asia (China, Korea, Japan)		Fang et al. 2000	AF145030
<i>L. godeffroyi</i>	F	Australia	Australia		Vink et al. 2002	AF380497
Genus <i>Notocosa</i>						
<i>N. bellinosa</i>	M	N. Zealand	New Zealand		Vink et al. 2002	AF380493
Genus <i>Pardosa</i>						
<i>P. astrigera</i>	M	Korea	Russia, Asia (China, Korea, Taiwan, Japan)		This study, Murphy et al. 2006	DQ173433
<i>P. brevitulva</i>	M	Korea	Asia (Korea, China, Japan)		This study, Murphy et al. 2006	DQ019783
<i>P. hedraii</i>	F	Korea	Russia, Asia (China, Korea, Japan)		This study, Murphy et al. 2006	DQ019795
<i>P. isago</i>	M	Korea	Russia, Asia (China, Korea, Japan)		This study, Murphy et al. 2006	DQ019796
<i>P. laura</i>	M	Korea	Russia, Asia (China, Korea, Japan)		This study, Murphy et al. 2006	DQ019797
<i>P. lugabris</i>	F	Korea	Russia, Asia (China, Korea, Japan, Taiwan)		This study, Murphy et al. 2006	DQ019797
<i>P. hyrtiferu</i> <sup>e</sup>	M	Korea	Palaearctic		This study, Murphy et al. 2006	DQ019798
<i>P. palustris</i>	M	Korea	Asia (China, Korea, Japan)		This study	DQ463222
<i>P. agrestis</i>	M	Korea	Holarctic		This study, Murphy et al. 2006	AJ008033
<i>P. alacris</i>	M	C. Europe	Palaearctic		Zehethofer and Sturmhuber 1998	AJ008028
		C. Europe	Europe, Russia		Zehethofer and Sturmhuber 1998	

Table 1. Continued

Species	Sex	Sampling locality <sup>a</sup>	Distribution <sup>b</sup>	Reference	Accession no.
<i>P. arenata</i>	1 M/2 F	C. Europe	Europe, Russia	Zehethofer and Sturmhuber 1998	AJ008023
<i>P. blanda</i>	M	C. Europe	Palaearctic	Zehethofer and Sturmhuber 1998	AJ008029
<i>P. hortensis</i>	M	C. Europe	Palaearctic	Zehethofer and Sturmhuber 1998	AJ008007
<i>P. lugubris</i>	1 M/1 F	C. Europe	Palaearctic	Zehethofer and Sturmhuber 1998	AJ008018
<i>P. nigra</i>	F	C. Europe	Palaearctic	Zehethofer and Sturmhuber 1998	AJ008008
<i>P. onophila</i>	M	C. Europe	Central, southern Europe	Zehethofer and Sturmhuber 1998	AJ008009
<i>P. paladivola</i>	M	C. Europe	Palaearctic	Zehethofer and Sturmhuber 1998	AJ008010
<i>P. palustris</i>	M	C. Europe	Holarctic	Zehethofer and Sturmhuber 1998	AJ008011
<i>P. pratvonga</i>	F	C. Europe	Europe, Russia	Zehethofer and Sturmhuber 1998	AJ008012
<i>P. pullata</i>	M	C. Europe	Europe, Russia, central Asia	Zehethofer and Sturmhuber 1998	AJ008014
<i>P. riparia</i>	M	C. Europe	Palaearctic	Zehethofer and Sturmhuber 1998	AJ008031
<i>P. saturator</i>	M	C. Europe	Central Europe	Zehethofer and Sturmhuber 1998	AJ008013
<i>P. takahashii</i>			Asia (China, Taiwan, Japan, Okinawa)	Fang et al. 2000	AF145052
Genus <i>Pirata</i>					
<i>P. piratoides</i>	F	Korea	Asia (China, Korea, Japan)	This study, Murphy et al. 2006	DQ019803
<i>P. subpiraticus</i>	F	Korea	Asia (China, Korea, Japan)	This study, Murphy et al. 2006	DQ019804
<i>P. hygrophilus</i>	M	C. Europe	Palaearctic	Zehethofer and Sturmhuber 1998	AJ008015
<i>P. knorri</i>	F	C. Europe	Palaearctic	Zehethofer and Sturmhuber 1998	AJ008019
Genus <i>Trochosa</i>					
<i>T. ruficollis</i>	F	Korea	Holarctic, Bermuda	This study, Murphy et al. 2006	DQ019814
<i>T. oraria</i>	F	Australia	Western Australia	Vink et al. 2002	AF380501
<i>T. spinipalpis</i>	F	C. Europe	Palaearctic	Zehethofer and Sturmhuber 1998	AJ008016
<i>T. terricola</i>	F	C. Europe	Holarctic	Zehethofer and Sturmhuber 1998	AJ008017
Genus <i>Varacosa</i>					
<i>V. azara</i>	M	USA	North America	Vink et al. 2002	AF380500
Genus <i>Venatrix</i>					
<i>V. goyderi</i>	F	N. Zealand	Australasia	Vink et al. 2002	AF380496
<i>V. lapidosa</i>	M	Australia	Australia	Vink et al. 2002	AF380495
Genus <i>Xeroligocosa</i>					
<i>X. nemoralis</i> <sup>d</sup>	M	Korea	Palaearctic	This study, Murphy et al. 2006	DQ019821
<i>X. miniata</i>	M	C. Europe	Palaearctic	Zehethofer and Sturmhuber 1998	AJ008020
<i>X. nemoralis</i>	M	C. Europe	Palaearctic	Zehethofer and Sturmhuber 1998	AJ008021
Genus <i>Dolomedes</i> (Pisauridae)					
<i>D. minor</i>	F	N. Zealand	New Zealand	Vink et al. 2002	AF380503
<i>D. raptor</i>			Russia, Asia (China, Korea, Japan)	Fang et al. 2000	AF145031

<sup>a</sup> C. Europe and N. Zealand indicate central Europe and New Zealand, respectively.

<sup>b</sup> Distributions are designated according to Platnick (2006).

<sup>c</sup> Currently listed as junior synonymy of *A. stigmosa*, but morphological evidence suggests it to be a valid species (J.-S.Y., unpublished data).

<sup>d</sup> Species indicate that samples used for sequencing were juveniles.

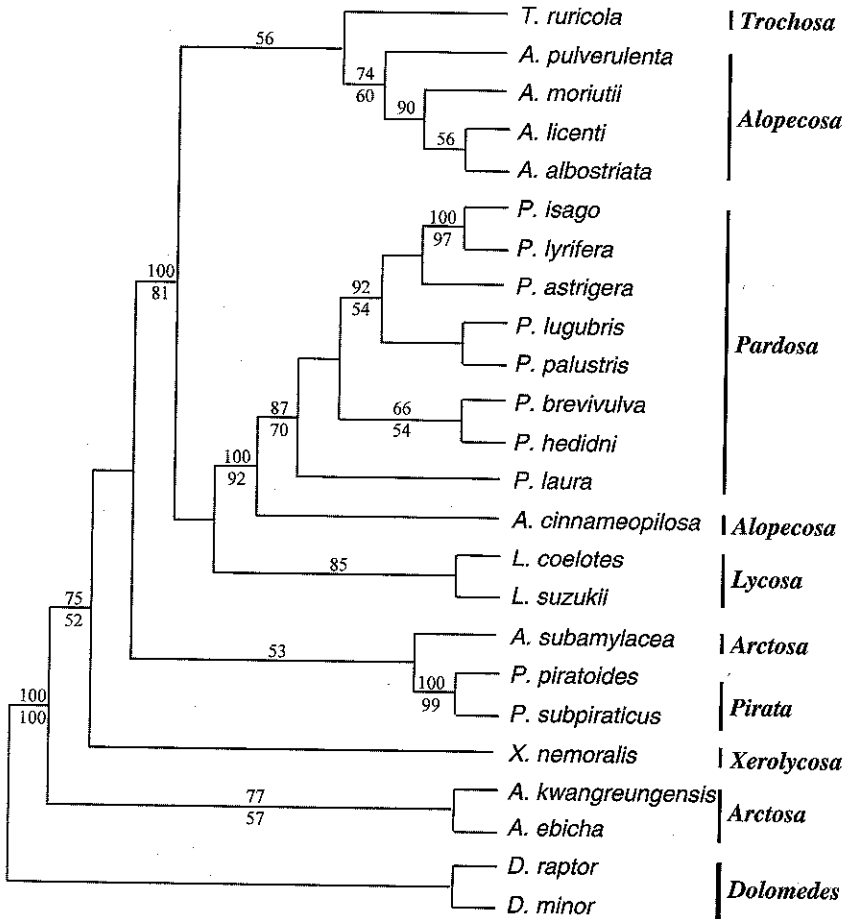


Fig. 1. Phylogeny of Korean lycosid spiders inferred from 12S rRNA gene sequences. The topology derived from BI analysis is given. The topologies of trees derived from ML and BI analyses did not markedly differ in relationships among key groups but estimates of node support were generally much lower for the ML analysis. In the BI analysis, the data set was treated as a single partition and a GTR model was specified as the most general model. PP, expressed as percentages, are shown above branches to indicate the level of support for each node. Branches with <50% PP were collapsed to form polytomies. Bootstrap values from the ML analysis are shown below branches. See Table 1 for details on the distribution of species and sampling localities.

tutional dynamics. We set variable rates as a prior and used the "adgamma" option, which assumes gamma distributed rates but with the possibility of autocorrelated rates. Felsenstein and Churchill (1996) argue that correlation of rates between adjacent sites is more realistic than uncorrelated rates, but we note that the autocorrelation parameter in our analyses can take a zero value. Otherwise we used the MrBayes default priors because these values are generally flat and uninformative.

Bayesian analyses were run for  $2 \times 10^6$  generations, sampling every 500th generation to reduce autocorrelation among parameter values across sampled generations. Stationarity in model parameters was checked using graphs of log likelihood values against generations sampled. We used a burning period of one million generations, well beyond the reaching of stationarity. The analysis was repeated three times to check for convergence of both consensus topology

and parameter estimates. Node support was assessed using posterior probabilities.

### Results and Discussion

**Phylogenetic Analysis of Korean Lycosid Spiders.** This is the first comprehensive study to infer molecular phylogenetic relationship for Korean wolf spiders. Topologies of trees derived from ML and BI analyses did not markedly differ in relationships among key groups (Fig. 1). However, estimates of node support were generally much lower for the ML analysis. This is similar to a variety of other studies (e.g., Wilcox et al. 2002, Cummings et al. 2003, Erixon et al. 2003) that show that bootstrapping generally leads to higher levels of support than posterior probability (PP) values. With the exception of two *Arctosa* spp. and *A. cinnameopilosa*, monophyly of each genus was moderately to highly supported in the BI analyses

(74–100%), although ML bootstrap support was comparatively lower (35–99%).

Our study includes eight of the 15 *Pardosa* species currently recognized in Korea (Kim et al. 2005). Monophyly of *Pardosa* was supported by relatively high bootstrap values, but the resolution was low within this clade. As Zehethofer and Sturmbauer (1998) suggested, this lack of resolution might result from recent history of *Pardosa* divergence and relatively rapid proliferation of the genus. The sequence alignment revealed that the *Pardosa isago* sequence did not differ from *P. lyrifera*. This could indicate conspecificity of both species, but an error in spider identification cannot be excluded because we used juveniles of these species for sequencing. *Pardosa* spp. grouped into three clades, but their supporting values are very weak (Fig. 1).

This study contains five of nine Korean *Alopecosa* species. Four of these grouped together (60% bootstrap support for ML; 74% PP support for BI). However, *A. cinnameopilosa* was recovered within a strongly supported monophyletic group that contained *Pardosa* species and the level of support for this was sufficiently high (92% bootstrap support for ML; 100% PP support for BI) to indicate an incorrect generic assignment of *A. cinnameopilosa*. *A. cinnameopilosa* differs from other *Alopecosa* spp. in some somatic structures, in particular its mottled abdominal pattern, which is much more similar to that of the genus *Pardosa* (Yoo 2001). Thus, we suggest transferring *A. cinnameopilosa* to the genus *Pardosa*, although further morphological studies are required to confirm this transfer. The clade including *Trochosa* spp. was a sister to that of *Alopecosa*, with weak supporting values.

Monophyly of the genus *Arctosa* was not supported. This genus includes two very distant clades: *Arctosa subamylacea* is a sister taxon to a clade including the two *Pirata* species, whereas the other two *Arctosa* species (*A. kwangreungensis* and *A. ebicha*) are a sister group to all the other Korean lycosid spiders.

**Phylogenetic Analysis of Lycosid Spiders in East Asia, Australasia, and Central Europe.** The combined data set was constructed from the sequences of 22 Asian wolf spider and those of previously reported 44 wolf spiders. As with analyses for the Korean taxa, ML bootstrap support values are generally much lower than PP values (Fig. 2). Some ML bootstrap values seem surprisingly low; for example, support for monophyly of *Pardosa* was 42% for the ML analysis, whereas it was 100% for the BI analysis. In the Bayesian analysis, monophyly was relatively well supported in the genera *Xerolycosa*, *Pirata*, *Anoteropsis*, *Pardosa*, and *Venatrix* (Fig. 2).

Support for monophyly of the three *Xerolycosa* species was high (94% bootstrap support and 100% PP support), regardless of the region they were collected in. A similar result was obtained for the genus *Pirata*: Korean and the European *Xerolycosa nemoralis* (Palearctic) grouped together. The two *X. nemoralis* and European *X. miniata* (Palearctic) also group well. Korean *Pirata* species of *Pirata subpiraticus* (Asia) and

*P. piratoides* (Asia) group well with the other two Palearctic species (*P. knorri* and *P. hygrophilus*). Despite wide distributional ranges, monophyly of *Pardosa* is well supported. Korean *Pardosa* species are scattered among various clades within *Pardosa*. *Pardosa laura* (Asia) from Korea and *P. takahashii* (Asia) group together. Korean *P. lugubris* (Palearctic) groups well with European *P. lugubris* and *P. alacris* (Russia and Europe), the type species of *Pardosa*. Korean *P. palustris* (Holarctic) and two European species of *P. blanda* (Palearctic) and *P. agrestis* (Palearctic) group into a single clade.

Monophyly of *Alopecosa* species, with the exception of *A. cinnameopilosa*, is well supported in Bayesian analysis (93% PP support for BI; Fig. 2), although its ML bootstrap value is low (Fig. 2). *Alopecosa cinnameopilosa* is firmly recovered as belonging to the *Pardosa* clade (100% PP support for BI). *Alopecosa* species within the genus form three groups whose Korean species (*A. moriutii*, *A. albostrigata*, and *A. licenti*) form a single group. *Trochosa* species of *T. rucicola* (Holarctic) from Korea, *T. terricola* (Holarctic) and *T. spinipalpis* (Palearctic) are well grouped together but the placement of the Australian *T. oraria* is ambiguous. This species has been argued to belong to a undescribed Australasian/Pacific subfamily including genera such as *Artoria*, *Diahogna*, and *Anoteropsis* (Framenau et al. 2006).

Within an overall phylogenetic framework of the Lycosidae, *Xerolycosa*, and *Pirata* were recovered in relatively more basal positions than all the remaining genera except *Arctosa kwangreungensis* and *A. ebicha*, but support for bifurcation order among these older divergences was low. The relationship between *Pirata* and *Xerolycosa* was well resolved in the previous studies on central European wolf spiders (Zehethofer and Sturmbauer 1998), suggesting that *Pirata* is more basal than *Xerolycosa*. However, in our analyses the relationship of the two still remains ambiguous, and although *Xerolycosa* was placed in a more basal position than *Pirata*, the bootstrap value was very low (<50%).

Our data set includes some previously published sequences of Australasian endemic genera. Spiders of the genus *Anoteropsis* and *Notocosa* are endemic to New Zealand (Vink 2002, Vink et al. 2002, Vink and Paterson 2003). *Artoria* are distributed widely in the Australasian region, including Australia, New Zealand, Papua New Guinea, and the Philippines (Framenau 2002, Vink 2002). Our results indicate that the monophyly of *Anoteropsis* is well supported, forming a sister clade to *Artoria* and *Notocosa* (73% bootstrap support for ML; 100% PP support for BI). Vink et al. (2002) noted that the placement of the three Australasian genera above was ambiguous within lycosid spiders. However, our Bayesian analysis indicates that the Australasian *Artoria* and *Anoteropsis* form a sister clade to the Palearctic species in the genus *Arctosa* (*A. subamylacea* and *A. leopardus*) (91% PP support for BI) and that these are the sister group to the Australian "*Trochosa*." This could suggest an Australasian origin for this group with a later dispersal into the Palearctic.

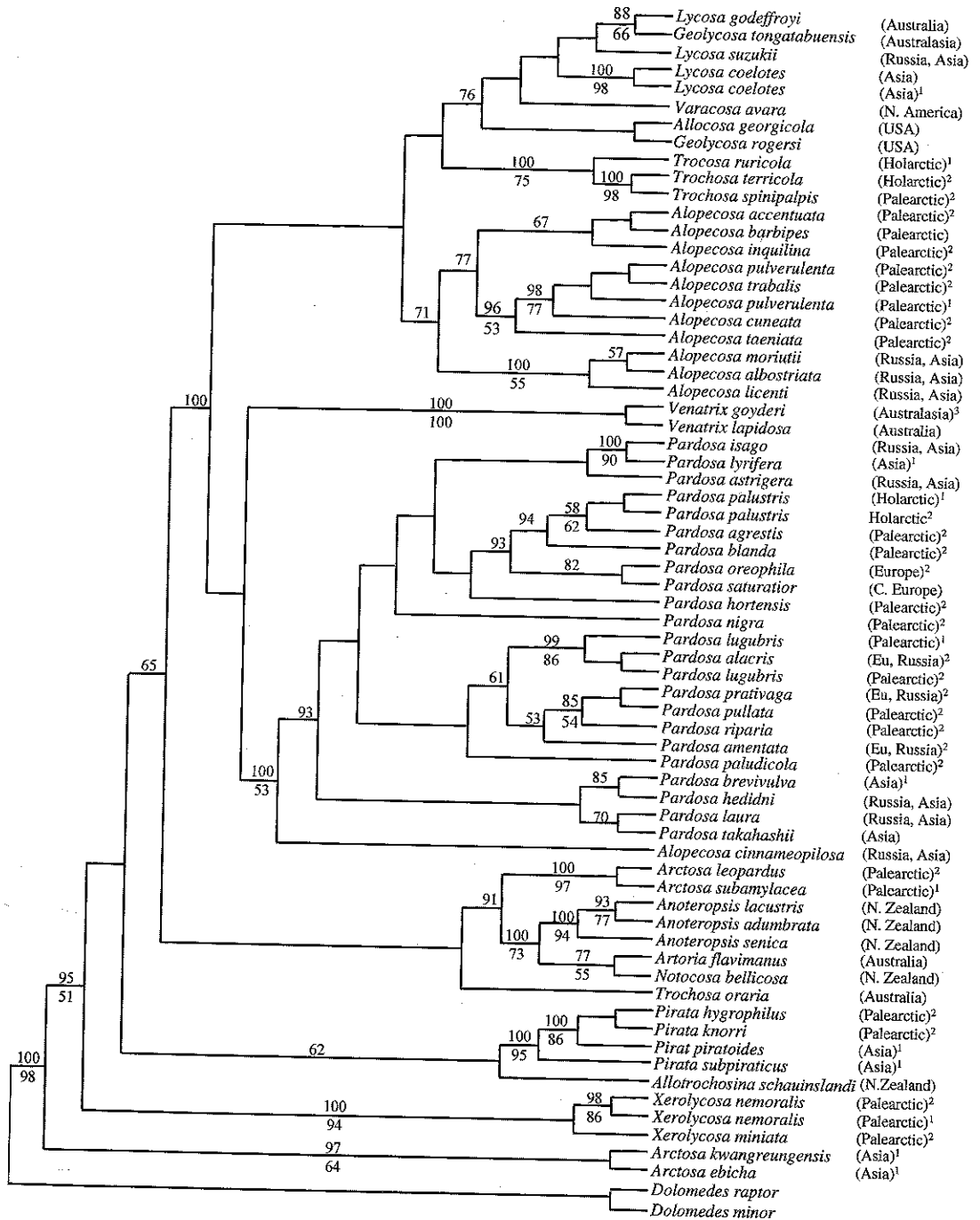


Fig. 2. Phylogenetic tree of lycosid spiders inferred from the combined data set of 22 sequences from Korean wolf spiders and 44 sequences previously reported from wolf spiders of Asia, Australasia, central Europe, and North America and two sequences from two outgroup spiders. The topology derived from BI analysis is given. As with the trees from the analyses for Korean taxa, the tree topologies derived from ML and BI analyses differed only in relationships among some terminal branches. However, estimates of node support were generally much lower for the ML analysis. In the BI analysis, the data set was treated as a single partition and a GTR model was specified as the most general model. PP, expressed as percentages, are shown above branches to indicate the level of support for each node. Bootstrap values from the ML analysis are shown below branches and they are expressed as percentages of 200 replicates. Species distributions based on Platnick (2006) are shown in parentheses on the right of terminal branches and the superscript numbers are the sampling localities (1, 2, and 3 indicate Korea and Japan, central Europe, and New Zealand, respectively). Eu and N. Zealand means Europe and New Zealand. See Table 1 for details on the distribution of species and sampling localities.



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