



## Molecular systematics of the wolf spider genus *Lycosa* (Araneae: Lycosidae) in the Western Mediterranean Basin

Enric Planas<sup>a</sup>, Carmen Fernández-Montraveta<sup>b</sup>, Carles Ribera<sup>a,\*</sup>

<sup>a</sup> Institut de Recerca de la Biodiversitat (IRBio), Departament de Biologia Animal, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain

<sup>b</sup> Departamento de Psicología Biológica y de la Salud, Universidad Autónoma de Madrid, Canto Blanco, 28049 Madrid, Spain

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### ABSTRACT

In this study, we present the first molecular phylogeny of the wolf spider genus *Lycosa* Latreille, 1804 in the Western Mediterranean Basin. With a wide geographic sampling comprising 90 localities and including more than 180 individuals, we conducted species delimitation analyses with a Maximum Likelihood approach that uses a mixed Yule-coalescent model to detect species boundaries. We estimated molecular phylogenetic relationships employing Maximum Likelihood and Bayesian Inference methods using mitochondrial and nuclear sequences. We conducted divergence time analyses using a relaxed clock model implemented in BEAST. Our results recovered 12 species that form four groups: *Lycosa tarantula* group comprising *L. tarantula* the type species of the genus, *L. hispanica* and *L. bedeli*; *Lycosa oculata* group composed of *L. oculata*, *L. suboculata* and three putative new species; *Lycosa baulnyi* group formed by the maghrebian *L. baulnyi* and *L. vachoni* and *Lycosa fasciiventris* group that includes two widespread species, *L. fasciiventris* and *L. munieri*. We found that each group of species shows a characteristic burrowing behavior and molecular and morphological diagnostic characters. Molecular clock analyses support the hypothesis of a relatively recent evolutionary origin of diversification of the group (4.96 Mya (3.53–6.45 Mya)). The establishment of the Mediterranean-like climate and the Pleistocenic glacial cycles seem to have been the main factors that promoted the diversification within the group. Finally, the results obtained in this study together with the revision of museum specimens, descriptions, redescrptions and illustrations, lead us to propose 18 nomenclatural changes (synonymies, generic transfers and *nomina dubia*) concerning the genera *Lycosa*, *Allocosa* and *Hogna* in the Western Mediterranean.

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### 1. Introduction

The wolf spider family (Lycosidae) is one of the most diverse spider families in the world, with 2393 described species grouped in 120 genera (Platnick, 2013). This high species diversity is fourth among all spider families; only the Salticidae, Linyphiidae and Araneidae are more diverse. The type genus of wolf spiders is *Lycosa* Latreille, 1804 comprising 223 extant species and five subspecies (Platnick, 2013) being one of the most species-rich genera within the lycosids.

In addition to their broad diversity, the Lycosidae are among the most paradigmatic spider species for many other reasons. Their world-wide distribution, relatively large body size, abundance and conspicuous synapomorphies, such as eye arrangement and brood care, make them well-known spiders even among the general public. Additionally, many researchers have chosen lycosid species as model organisms for ecological and behavioral studies

(e.g. Stratton and Uetz, 1983; Jakob et al., 1996; Kotiaho et al., 1996; McClintock and Uetz, 1996; Toft and Wise, 1999; Persons et al., 2001; Walker and Rypstra, 2003; Hebets, 2003; Hebets and Papaj, 2005; Hoefler et al., 2008; Wilder and Rypstra, 2010). This is especially true for the *Lycosa* species, which have been widely used as model organisms for studies of cannibalistic behavior (Moya-Laraño et al., 2004; Rabaneda-Bueno et al., 2008), territorial behavior (Moya-Laraño et al., 2002), communication (Tietjen, 1978; Moya-Laraño et al., 2003; Fernández-Montraveta and Cuadrado, 2009), sexual size dimorphism (Fernández-Montraveta and Moya-Laraño, 2007), ecology (Moya-Laraño, 2002; Moya-Laraño et al., 2008, de Almeida et al., 2010), antipredatory behavior (Williams et al., 2006), homing behavior (Ortega-Escobar, 2002, 2006, 2011, Reyes-Alcubilla et al., 2009) and venom composition (Zhang et al., 2010).

The extensive knowledge of these aspects of the genus contrasts with its controversial taxonomic status (Barrientos, 1981, 2004). The diagnosis of the genus *Lycosa* is poorly defined, and this genus has hence been used as a “dumping ground” for wolf spider species that could not be satisfactorily placed in other genera, resulting in a clearly polyphyletic genus (Murphy et al., 2006) with high cur-

\* Corresponding author. Fax: +34 3403 57 40.

E-mail address: [cribera@ub.edu](mailto:cribera@ub.edu) (C. Ribera).

rent genus diversity. The last attempt to redefine the genus *Lycosa* was carried out by Zyuzin and Logunov (2000), but despite its advancements, namely in including genitalia characters for the first time, this work was mainly focused on European representatives and did not include most Western Mediterranean species, among which the vast majority of the diversity in this group is found.

There are many reasons for the controversial taxonomic status of the *Lycosa* species. 1 – Old and inaccurate descriptions: Most of the original descriptions of these species date from the 18th, 19th and the first half of the 20th century, and they are often insufficiently accurate and poorly illustrated. 2 – Loss of type specimens: The loss of the type material for the majority of species impedes or seriously obstructs the correct identification at the species level. 3 – Poor and polymorphic diagnostic characters: In the second half of 20th century, Roewer carried out a taxonomic revision of the Lycosidae (Roewer, 1955, 1959) and transferred many “true” *Lycosa* species from the Western Mediterranean to the genus *Allocosa* Banks, 1900 and *Hogna* Simon, 1885, resulting in an even greater confusion concerning the delimitation of lycosid genera (Vink et al., 2002; Zyuzin and Logunov, 2000). This taxonomic classification was ill defined partly because much more relevance was given to adaptive morphological (coloration patterns, slight variation in the relative eye sizes) or behavioral (burrowing versus non-burrowing species) characters than to genitalic characters (cf. Dondale and Redner, 1983), which are commonly used to establish a reliable spider taxonomy (e.g. Huber, 2003). 4 – Partial geographic revisions: The most recent revision of the group in the study area was published by Guy (1966), who focused on the Lycosidae from northwestern Africa with a special emphasis on Moroccan fauna. He also made an attempt to delimit the genera found in this area, but did not consider the sexual characters of both sexes, leading to the same problems encountered by Roewer (1955, 1959). However, his work included new species descriptions and provided updated species range information. Recently, revisions of wolf spider systematics have redefined several genera and transferred numerous species from *Lycosa* to Australasian genera (e.g. Framenau, 2002; Langlands and Framenau, 2010), a process that has been performed for North American genera (Dondale and Redner, 1983; Brady, 2012) but not for the Mediterranean genera.

In this work, we focus on *Lycosa* species from the Western Mediterranean. In addition to the currently accepted species included in the genus, we also include species that were transferred by Roewer (1955, 1959) to the genera *Allocosa* and *Hogna*. This is necessary because the taxonomic instability of this genus has led subsequent authors to disregard the original “specific names” of species that Roewer (1955, 1959) transferred to other genera, describing new species and/or using the incorrect species names (see Logunov, 2011). As a clear example of this problematic taxonomic situation, according to the literature, there are 10 species of the genus *Allocosa* (and no representatives of the genus *Lycosa*) in Morocco, while in its neighboring country, Algeria, there are eight *Lycosa* species and only one *Allocosa* (Platnick, 2013). There are currently 36 accepted species belonging to these genera that putatively belong to the genus *Lycosa*. In western Europe, including the islands of Majorca, Corsica, Sardinia and Sicily, 13 valid species have been listed (Platnick, 2013); 5 of these were described by Franganillo (1918, 1920, 1925, 1926), and the type specimens were subsequently lost. The number of species is even higher in the southwestern part of the Mediterranean basin. Overall, 24 species belonging to these three genera have been described or recorded from the Maghreb (Tunisia, Algeria and Morocco) (Platnick, 2013), some of which were described on the basis of juvenile specimens and are of doubtful reliability.

There have been few molecular studies on lycosids, and those that have been performed focused mostly on Australasian genera (e.g. Vink et al., 2002; Murphy et al., 2006; Gotch et al., 2008), but European (e.g. Zehethofer and Sturmbauer, 1998; Muster and Berendonk, 2006) and Asian genera (e.g. Chang et al., 2007; Park et al., 2007) have also been studied. In most cases, these studies demonstrate the problematic status of the current taxonomy and the lack of useful characters for distinguishing genera and closely related species (Vink et al., 2002). In cases where traditional taxonomy has been unable to provide an accurate delimitation of species boundaries, DNA-based species delimitation methodologies, such as the General Mixed Yule-coalescent (GMYC), could be an alternative tool for recognizing species entities (Pons et al., 2006).

*Lycosa* species are important representatives of the soil fauna and constitute one of the larger groups present in semiarid ecosystems. Some authors have suggested that the Lycosidae are a family of recent evolutionary origin (Alderweireldt and Jocqué, 1992; Wunderlich, 2004; Jocqué and Alderweireldt, 2005) that co-evolved with grasslands and dispersed as this type of habitat expanded (Jocqué and Alderweireldt, 2005). Mediterranean ecosystems seem to have been established approximately 3.5 Mya (Estabrook, 2001; Blondel et al., 2010; Thompson, 2005), after a transition period from a subtropical to a typical Mediterranean-type climate, with hot, dry summers and mild winters that began approximately 3.9 Mya. A second period with strong consequences on the Mediterranean biota was initiated approximately 2.7 Mya by an abrupt climate shift toward more arid and cool conditions, i.e., the onset of the Northern Hemisphere glaciations period. These climatic transitions (deMenocal, 1995; Lisiecki and Raymo, 2007) had an important effect on ecosystems and have been the major climatic diversity drivers in the Mediterranean Basin (Coope, 1994; Taberlet et al., 1998; Veith et al., 2003). Providing a temporal framework for the resulting speciation processes would allow us to explore the causes that have contributed to the current diversity of this genus in the Western Mediterranean.

The spiders of the species-rich genus *Lycosa* are important components within the semiarid ecosystems of the Mediterranean Basin. It is crucial to obtain a phylogenetic framework to understand how the studied characteristics have arisen and to study their biogeographic history. A robust and reliable taxonomy of the wolf spider genus *Lycosa* is thus necessary and is expected to further facilitate such research. We conducted this study with the specific aims of (1) inferring a phylogeny for *Lycosa* species in the Western Mediterranean, (2) delimiting species boundaries using molecular data and (3) providing a temporal framework to shed light on the patterns and processes involved in the diversification of this genus.

## 2. Materials and methods

### 2.1. Taxon sampling and species-level identification

During 2002–2012, we sampled 90 localities (Fig. 1) across all the main areas of the Western Mediterranean: the Iberian Peninsula (PI), the Mediterranean coast of France (FR), the Italian Peninsula (IT), Sardinia (SA), the Balearic Islands (IB), Morocco (MA) and Tunisia (TN). Most of the sampling efforts were conducted at night, during the active period of *Lycosa*, or by visual inspection of suspected favorable zones during the day. Spiders were collected and directly preserved in 96% ethanol. One leg was removed shortly after collection to ensure the preservation of the DNA. A list of samples used and their corresponding localities can be found in Table 1. *Hogna radiata* (Latreille, 1817), *Arctosa alluaudi* Guy, 1966 and *Pisaura mirabilis* (Clerck, 1757) were used as outgroups.

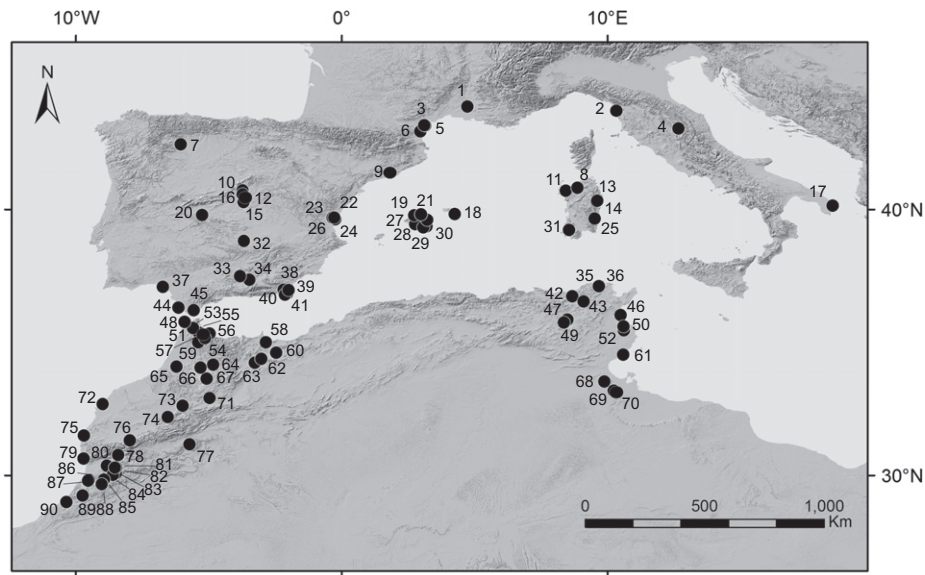


Fig. 1. Sampling localities of the *Lycosa* specimens used in this study. Locality numbers refer to specimens in Fig. 3 and Table 1.

For identification at the species level, we revised the *Lycosa* specimens deposited in the collections from the Museum National d'Histoire Naturelle of Paris (MNHN) and the male palp of *L. subhirsuta* Koch, 1881 (syntype) from the Senckenberg Museum of Frankfurt (SMF). We were not able to consult the collection deposited by Guy at the former Institut Scientifique Cherifien of Rabat (now Institut Scientifique de l'Université Mohammed V Agdal) due to its lack of accessibility. We reviewed the original descriptions, redescrptions and illustrations from different authors that provided information about the currently accepted *Lycosa* species. We have also revised Franganillo's collection, which is deposited at the Departamento de Biología de Organismos y Sistemas (BOS) of the Universidad de Oviedo (Spain). We found three tubes containing *Lycosa hispanica* Simon, 1870 specimens; two were not labeled, and the third was marked simply as "Granada" (either the city or the province). There was no reference to the collection number or species name for any of the three samples.

## 2.2. DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from the severed legs using the QIAamp® DNA Mini Kit (QIAGEN) or SPEEDTOOLS® Tissue DNA Extraction Kit (BIOTOOLS) following the manufacturers' protocol. Four gene regions were amplified, namely three mitochondrial fragments including portions of five genes (cytochrome *c* oxidase subunit 1, *cox1*; end of large ribosomal unit plus the Leucine transfer plus the 3' end of NADH dehydrogenase, 16S, +tRNA-Leu + *nadh*; a fragment of the small ribosomal unit, 12S) and a fragment of the large ribosomal unit of the nuclear genome (28S). The primers and conditions used are listed in Table 2. PCR reactions were carried out in a final volume of 25 µL using either *Taq* polymerase (Promega) or Biotools *Pfu* DNA Polymerase (Biotools). PCR products were cycle-sequenced in both directions using the same PCR primers and BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems) and analyzed in an ABI 3700 automated sequencer at the Serveis Científico-Tècnics of the Universitat de Barcelona. Raw sequences were edited and assembled with GENEIOUS v. 4.6.5 (Drummond et al., 2009). All the sequences used in this study are new and have been deposited in GenBank under the accession numbers KC550639–KC551233 (see Table 1).

## 2.3. Alignment and evolutionary model selection

The DNA sequences were aligned using the online version of MAFFT (Katoh et al., 2005) applying the G i-INS-i algorithm (Katoh et al., 2005) for protein coding fragments and Q-INS-i (Katoh and Toh, 2008) for the ribosomal gene fragments. The protein coding sequences were translated into amino acids, and no stop codons were found. We paid special attention to the results of the 28S analysis, as paralogous copies have been inferred in Lycosidae (Murphy et al., 2006) and Salticidae (Vink et al., 2011). In the present study, no signal of amplification of paralogous copies was detected. Evolutionary models for each partition were estimated with jModeltest v. 0.1.1 (Posada, 2008) and selected under the Akaike Information Criterion (AIC) (Akaike, 1974).

## 2.4. Molecular species delimitation

We used the DNA-based approach proposed by Pons et al. (2006) implemented in the R package SPLITS (Ezard et al., 2009). This method detects the transition in the rate of lineage branching of an ultrametric tree from interspecific to intraspecific branching and identifies clusters of specimens corresponding to putative species. Two models are implemented in a likelihood framework to account for the branching process of the entire tree. Under the null model, the whole sample derives from a single population obeying a coalescent process. The alternative model, called a General Mixed Yule-coalescent (GMYC) model, combines equations that separately describe branching within populations (coalescent process) and branching between species (a Yule model). Under the GMYC model, a threshold (T) is optimized such that nodes before the threshold are considered species diversification events, and branches crossing the threshold define clusters following a coalescent process. A standard likelihood ratio test (LRT) was used to assess whether the alternative model provides a better fit than the null model. If the GMYC model is favored over the null model, the T parameter of the Maximum Likelihood solution allows the number of species to be estimated. To estimate species number, we generated an ultrametric tree using BEAST v. 1.6.2 (Drummond and Rambaut, 2007) with a dataset containing mitochondrial sequences for all sequenced individuals partitioned by gene fragment (*cox1*, 16S-tRNA-Leu, *nadh* and 12S). We performed two independent runs of 30 million generations each leaving all priors at the

Table 1

List of specimens included in this study, with their identification code, locality and Genbank accession numbers.

Code <sup>a</sup>	Genus	Species	Locality	L. N. <sup>b</sup>	Lat_Long	COI	12S	16S	28S
LC1002_TN	<i>Lycosa</i>	<i>bedeli</i>	Hdadra	50	35.613 N 10.602 E	KC550713	KC551047	KC551195	KC550831
LC1022_TN	<i>Lycosa</i>	<i>bedeli</i>	Hdadra	50	35.613 N 10.602 E	KC550714	KC551053	KC551197	
LC1068_TN	<i>Lycosa</i>	<i>bedeli</i>	Mastouta	43	36.54 N 9.097 E	KC550715	KC550991	KC551196	KC550839
LC1398_MA	<i>Lycosa</i>	<i>bedeli</i>	Gouttitr	62	34.386 N 3.013 W	KC550716	KC550926	KC551198	KC550889
LC1402_MA	<i>Lycosa</i>	<i>bedeli</i>	El Aioun	60	34.627 N 2.459 W	KC550719	KC550942	KC551200	
LC1404_MA	<i>Lycosa</i>	<i>bedeli</i>	El Aioun	60	34.627 N 2.459 W	KC550717	KC550939	KC551201	KC550892
LC1412_MA	<i>Lycosa</i>	<i>bedeli</i>	Agalane	58	35.015 N 2.842 W	KC550718	KC550940	KC551199	KC550893
LC1087_IT	<i>Lycosa</i>	<i>tarantula</i>	Otranto	17	40.148 N 18.468 E	KC550665	KC550997	KC551204	
LC1088_IT	<i>Lycosa</i>	<i>tarantula</i>	Otranto	17	40.148 N 18.468 E	KC550669	KC551085	KC551206	KC550842
LC1089_IT	<i>Lycosa</i>	<i>tarantula</i>	Otranto	17	40.148 N 18.468 E	KC550666	KC551036	KC551202	
LC1090_IT	<i>Lycosa</i>	<i>tarantula</i>	Sant Rossore	2	43.72 N 10.337 E	KC550667	KC551084	KC551205	KC550843
LC1091_IT	<i>Lycosa</i>	<i>tarantula</i>	Sant Rossore	2	43.72 N 10.337 E	KC550668	KC551015	KC551207	
LC1586_IT	<i>Lycosa</i>	<i>tarantula</i>	Assisi	4	43.053 N 12.676 E		KC550950		
LCFRA6_FR	<i>Lycosa</i>	<i>tarantula</i>	Le Gard	1	43.875 N 4.727 E	KC550663	KC551028	KC551203	KC550915
LCFRA7_FR	<i>Lycosa</i>	<i>tarantula</i>	Le Gard	1	43.875 N 4.727 E	KC550664			
AL1182_PI	<i>Lycosa</i>	<i>hispanica</i>	Los Barrios	45	36.221 N 5.558 W	KC550639	KC551074		KC550818
AL1184_PI	<i>Lycosa</i>	<i>hispanica</i>	La Boca de los Frailes	41	36.806 N 2.133 W	KC550640	KC550968		KC550819
AL9921_PI	<i>Lycosa</i>	<i>hispanica</i>	La Boca de los Frailes	41	36.805 N 2.132 W	KC550641	KC551068		KC550821
CO0086_PI	<i>Lycosa</i>	<i>hispanica</i>	Tres Cantos	12	40.55 N 3.708 W	KC550646	KC551063		KC550824
CO9932_PI	<i>Lycosa</i>	<i>hispanica</i>	Tres Cantos	12	40.55 N 3.708 W	KC550647	KC550981		
CP1193_PI	<i>Lycosa</i>	<i>hispanica</i>	Soto del Real	10	40.727 N 3.727 W	KC550648	KC551031		KC550825
CP1266_PI	<i>Lycosa</i>	<i>hispanica</i>	Soto del Real	10	40.727 N 3.727 W	KC550649	KC551076		
CR1129_PI	<i>Lycosa</i>	<i>hispanica</i>	Ciudad Real	32	38.823 N 3.673 W	KC550653	KC550983		KC550826
CR1131_PI	<i>Lycosa</i>	<i>hispanica</i>	Ciudad Real	32	38.823 N 3.673 W	KC550654	KC551067		
DO1162_PI	<i>Lycosa</i>	<i>hispanica</i>	Huelva	37	37.092 N 6.716 W	KC550645	KC551012		KC550827
LC1115_MA	<i>Lycosa</i>	<i>hispanica</i>	Madissou	57	35.174 N 5.139 W	KC550661	KC550969	KC551216	KC550845
LC1223_PI	<i>Lycosa</i>	<i>hispanica</i>	Unknown	91	Unknown	KC550657	KC551061	KC551215	KC550861
LC1344_PI	<i>Lycosa</i>	<i>hispanica</i>	Carboneras	39	36.967 N 1.99 W	KC550658	KC550977	KC551208	KC550877
LC1472_PI	<i>Lycosa</i>	<i>hispanica</i>	Iznalloz	34	37.366 N 3.469 W	KC550660	KC551050	KC551210	
LC1473_PI	<i>Lycosa</i>	<i>hispanica</i>	Pedrero, Léon	7	42.45 N 6.05 W	KC550659	KC551018	KC551213	KC550899
LC1474_PI	<i>Lycosa</i>	<i>hispanica</i>	Pedrero, Léon	7	42.45 N 6.05 W	KC550643	KC550998	KC551214	
LC1504_PI	<i>Lycosa</i>	<i>hispanica</i>	Frailes	33	37.491 N 3.824 W	KC550642	KC550966	KC551212	KC550902
LCGARR_PI	<i>Lycosa</i>	<i>hispanica</i>	Garraf	9	Unknown	KC550662	KC551072	KC551211	KC550916
LCYG01_PI	<i>Lycosa</i>	<i>hispanica</i>	Getafe	15	40.29 N 3.685 W	KC550656	KC551065		KC550919
LCYG02_PI	<i>Lycosa</i>	<i>hispanica</i>	Getafe	15	40.29 N 3.685 W	KC550651	KC550979		
RO1148_PI	<i>Lycosa</i>	<i>hispanica</i>	Roche	44	36.318 N 6.139 W	KC550644	KC550951	KC551209	KC550920
TO1167_PI	<i>Lycosa</i>	<i>hispanica</i>	Valdeverdeja	20	39.798 N 5.247 W	KC550655	KC550949		KC550921
TR1109_PI	<i>Lycosa</i>	<i>hispanica</i>	Tres Cantos	12	40.578 N 3.721 W	KC550650	KC550947		
TR1171_PI	<i>Lycosa</i>	<i>hispanica</i>	Tres Cantos	12	40.578 N 3.721 W	KC550652	KC551034		
LC1403_MA	<i>Lycosa</i>	<i>aff. oculata</i> n. sp. 1	El Aioun	60	34.627 N 2.459 W	KC550679	KC550943	KC551226	KC550891
LC1413_MA	<i>Lycosa</i>	<i>aff. oculata</i> n. sp. 1	Agalane	58	35.015 N 2.842 W	KC550680	KC550944	KC551229	KC550894
LC1414_MA	<i>Lycosa</i>	<i>aff. oculata</i> n. sp. 1	Agalane	58	35.015 N 2.842 W	KC550681	KC550935	KC551228	
LC1415_MA	<i>Lycosa</i>	<i>aff. oculata</i> n. sp. 1	Agalane	58	35.015 N 2.842 W	KC550682	KC550948	KC551227	
LC1598_MA	<i>Lycosa</i>	<i>aff. oculata</i> n. sp. 2	Near Fort Bou-Jerif	90	29.011 N 10.345 W	KC550683		KC551230	
LC1603_MA	<i>Lycosa</i>	<i>aff. oculata</i> n. sp. 2	Near Fort Bou-Jerif	90	29.011 N 10.345 W	KC550684	KC551002	KC551231	KC550909
LC1000_TN	<i>Lycosa</i>	<i>oculata</i>	Hdadra	50	35.62 N 10.6 E	KC550670	KC550978	KC551220	KC550829
LC1012_TN	<i>Lycosa</i>	<i>oculata</i>	Hdadra	50	35.62 N 10.6 E	KC550671	KC551013	KC551217	
LC1013_TN	<i>Lycosa</i>	<i>oculata</i>	Hdadra	50	35.62 N 10.6 E	KC550674	KC551087	KC551225	
LC1018_TN	<i>Lycosa</i>	<i>oculata</i>	Hdadra	50	35.62 N 10.6 E	KC550672	KC550955	KC551219	
LC1019_TN	<i>Lycosa</i>	<i>oculata</i>	Hdadra	50	35.62 N 10.6 E	KC550673	KC551032	KC551218	
LC1064_TN	<i>Lycosa</i>	<i>oculata</i>	Hergla	46	36.037 N 10.492 E	KC550678	KC550941	KC551224	KC550837
LC1303_SA	<i>Lycosa</i>	<i>oculata</i>	Laerru	8	40.823 N 8.871 E	KC550675	KC551044	KC551221	
LC1309_SA	<i>Lycosa</i>	<i>oculata</i>	Laerru	8	40.823 N 8.871 E	KC550677	KC551078	KC551223	
LC1311_SA	<i>Lycosa</i>	<i>oculata</i>	Laerru	8	40.823 N 8.871 E	KC550676	KC550994	KC551222	KC550869
LC1096_MA	<i>Lycosa</i>	<i>aff. suboculata</i> n. sp.	Sagor	81	30.302 N 8.52 W	KC550685	KC551059	KC551173	
LC1154_MA	<i>Lycosa</i>	<i>aff. suboculata</i> n. sp.	Tagalmoust	86	29.807 N 9.53 W	KC550695	KC550970	KC551178	KC550849
LC1164_MA	<i>Lycosa</i>	<i>aff. suboculata</i> n. sp.	Tagalmoust	86	29.807 N 9.53 W	KC550688	KC550975		
LC1166_MA	<i>Lycosa</i>	<i>aff. suboculata</i> n. sp.	Tafroute	88	29.681 N 9.023 W	KC550691	KC550995		
LC1167_MA	<i>Lycosa</i>	<i>aff. suboculata</i> n. sp.	Tafroute	88	29.681 N 9.023 W	KC550692	KC550993	KC551179	KC550851
LC1169_MA	<i>Lycosa</i>	<i>aff. suboculata</i> n. sp.	Sagor	81	30.302 N 8.52 W	KC550693	KC550990		KC550853
LC1171_MA	<i>Lycosa</i>	<i>aff. suboculata</i> n. sp.	Tafroute	88	29.681 N 9.023 W	KC550694	KC550985		KC550855
LC1174_MA	<i>Lycosa</i>	<i>aff. suboculata</i> n. sp.	Tafroute	88	29.681 N 9.023 W	KC550689	KC551023	KC551175	
LC1201_MA	<i>Lycosa</i>	<i>aff. suboculata</i> n. sp.	Tafroute	88	29.681 N 9.023 W	KC550690	KC551022	KC551174	
LC1221_MA	<i>Lycosa</i>	<i>aff. suboculata</i> n. sp.	Tafroute	88	29.681 N 9.023 W	KC550686	KC551035	KC551176	
LC1362_MA	<i>Lycosa</i>	<i>aff. suboculata</i> n. sp.	Arhoud	79	30.643 N 9.706 W	KC550687	KC550959	KC551177	KC550880
LC1224_MA	<i>Lycosa</i>	<i>suboculata</i>	Douar Nzala	66	34.061 N 5.302 W	KC550696	KC551000	KC551183	KC550862
LC1265_MA	<i>Lycosa</i>	<i>suboculata</i>	Douar Nzala	66	34.061 N 5.302 W	KC550709	KC550953	KC551185	
LC1269_MA	<i>Lycosa</i>	<i>suboculata</i>	Douar Nzala	66	34.061 N 5.302 W	KC550712	KC550954	KC551188	
LC1358_MA	<i>Lycosa</i>	<i>suboculata</i>	Oualidia	72	32.689 N 8.984 W	KC550697	KC551081	KC551192	KC550878
LC1359_MA	<i>Lycosa</i>	<i>suboculata</i>	Oualidia	72	32.689 N 8.984 W	KC550698	KC550946	KC551193	
LC1360_MA	<i>Lycosa</i>	<i>suboculata</i>	Afrate Haïsioun	75	31.505 N 9.696 W	KC550699	KC551090		KC550879
LC1361_MA	<i>Lycosa</i>	<i>suboculata</i>	Afrate Haïsioun	75	31.505 N 9.696 W	KC550700	KC551026	KC551191	
LC1380_MA	<i>Lycosa</i>	<i>suboculata</i>	Imouzer Kandar	67	33.644 N 5.069 W	KC550701		KC551180	KC550886
LC1383_MA	<i>Lycosa</i>	<i>suboculata</i>	Douar Nzala	66	34.059 N 5.303 W	KC550702	KC550934	KC551194	KC550887

(continued on next page)

Table 1 (continued)

Code <sup>a</sup>	Genus	Species	Locality	L. N. <sup>b</sup>	Lat_Long	COI	12S	16S	28S
LC1427_MA	<i>Lycosa</i>	<i>suboculata</i>	Douar Grazen	59	35.01 N 5.394 W	KC550703		KC551181	KC550895
LC1430_MA	<i>Lycosa</i>	<i>suboculata</i>	Fôret Maamora	65	34.092 N 6.202 W	KC550705		KC551184	KC550896
LC1431_MA	<i>Lycosa</i>	<i>suboculata</i>	Fôret Maamora	65	34.092 N 6.202 W	KC550704	KC550928	KC551187	
LC1434_MA	<i>Lycosa</i>	<i>suboculata</i>	Fôret Maamora	65	34.092 N 6.202 W	KC550706		KC551182	
LC1437_MA	<i>Lycosa</i>	<i>suboculata</i>	Fôret Maamora	65	34.092 N 6.202 W	KC550707		KC551189	
LC1438_MA	<i>Lycosa</i>	<i>suboculata</i>	Fôret Maamora	65	34.092 N 6.202 W	KC550710	KC550937		
LC1439_MA	<i>Lycosa</i>	<i>suboculata</i>	Fôret Maamora	65	34.092 N 6.202 W	KC550711		KC551186	
LC1445_MA	<i>Lycosa</i>	<i>suboculata</i>	Fôret Maamora	65	34.092 N 6.202 W	KC550708		KC551190	
LC1007_TN	<i>Lycosa</i>	<i>vachoni</i>	Hdadra	50	35.613 N 10.602 E	KC550773	KC551029	KC551141	
LC1008_TN	<i>Lycosa</i>	<i>vachoni</i>	Kerker	52	35.461 N 10.606 E	KC550775	KC551088	KC551146	KC550833
LC1016_TN	<i>Lycosa</i>	<i>vachoni</i>	Hdadra	50	35.62 N 10.6 E	KC550777	KC551003	KC551143	
LC1017_TN	<i>Lycosa</i>	<i>vachoni</i>	Hdadra	50	35.62 N 10.6 E	KC550779	KC551021	KC551144	KC550834
LC1063_TN	<i>Lycosa</i>	<i>vachoni</i>	Hergla	46	36.037 N 10.492 E	KC550781	KC551009	KC551145	
LC1065_TN	<i>Lycosa</i>	<i>vachoni</i>	Hergla	46	36.037 N 10.492 E	KC550778	KC551024	KC551142	KC550838
LC1066_TN	<i>Lycosa</i>	<i>vachoni</i>	Hergla	46	36.037 N 10.492 E	KC550776	KC551089	KC551147	
LC1609_TN	<i>Lycosa</i>	<i>vachoni</i>	Temezret dir. Matmata	68	33.539 N 9.887 E	KC550774	KC551049		KC550911
LC1610_TN	<i>Lycosa</i>	<i>vachoni</i>	Beni Kheddoche	69	33.211 N 10.24 E	KC550780	KC550962		KC550912
LC1611_TN	<i>Lycosa</i>	<i>vachoni</i>	Krerachfa	70	33.193 N 10.235 E	KC550811	KC550964		KC550913
LC1612_TN	<i>Lycosa</i>	<i>vachoni</i>	Naktar	61	34.55 N 10.593 E		KC550987		KC550914
LC1095_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tafroute	88	29.681 N 9.023 W	KC550782	KC551055	KC551150	
LC1097_MA	<i>Lycosa</i>	<i>baulnyi</i>	Sagor	81	30.302 N 8.52 W	KC550796	KC551001	KC551167	
LC1104_MA	<i>Lycosa</i>	<i>baulnyi</i>	Irherm	82	30.092 N 8.488 W	KC550783	KC551069	KC551158	
LC1152_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tagalmoust	87	29.839 N 9.498 W	KC550802	KC550960	KC551161	KC550848
LC1158_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tafroute	88	29.681 N 9.023 W	KC550807	KC550956		
LC1160_MA	<i>Lycosa</i>	<i>baulnyi</i>	Irherm	82	30.092 N 8.488 W	KC550804	KC551008	KC551153	KC550850
LC1165_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tagalmoust	87	29.839 N 9.498 W	KC550809	KC551041	KC551155	
LC1170_MA	<i>Lycosa</i>	<i>baulnyi</i>	Taroudant	80	30.371 N 8.83 W	KC550808	KC551091		KC550854
LC1188_MA	<i>Lycosa</i>	<i>baulnyi</i>	Sagor	81	30.302 N 8.52 W	KC550806	KC550989	KC551154	KC550856
LC1192_MA	<i>Lycosa</i>	<i>baulnyi</i>	Bouizakarne	89	29.249 N 9.743 W	KC550805	KC551043	KC551157	KC550857
LC1195_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tahnaout	76	31.318 N 7.956 W	KC550792	KC551046	KC551156	KC550858
LC1199_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tafroute	88	29.681 N 9.023 W	KC550810	KC551020	KC551148	KC550859
LC1210_MA	<i>Lycosa</i>	<i>baulnyi</i>	Irherm	82	30.092 N 8.488 W	KC550784	KC551073	KC551165	
LC1215_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tafroute	88	29.681 N 9.023 W	KC550785	KC551077	KC551149	
LC1222_MA	<i>Lycosa</i>	<i>baulnyi</i>	Oualegh	71	32.921 N 4.963 W	KC550797	KC551025	KC551171	KC550860
LC1365_MA	<i>Lycosa</i>	<i>baulnyi</i>	Sagor	81	30.297 N 8.474 W	KC550786	KC551057	KC551159	
LC1366_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tagdir Nait Ali	83	30.025 N 8.59 W	KC550787	KC551010	KC551166	KC550881
LC1368_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tizgzaouin	85	29.846 N 8.938 W	KC550788	KC550922	KC551166	KC550883
LC1370_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tajgalt	78	30.774 N 8.402 W	KC550789	KC550923	KC551163	KC550884
LC1373_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tajgalt	78	30.774 N 8.402 W	KC550790	KC550932		
LC1374_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tajgalt	78	30.774 N 8.402 W	KC550791		KC551160	
LC1375_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tajgalt	78	30.774 N 8.402 W	KC550795	KC550930	KC551164	
LC1389_MA	<i>Lycosa</i>	<i>baulnyi</i>	Guercif	63	34.248 N 3.265 W	KC550798	KC550925	KC551170	KC550888
LC1391_MA	<i>Lycosa</i>	<i>baulnyi</i>	Guercif	63	34.248 N 3.265 W	KC550800	KC550933	KC551168	
LC1393_MA	<i>Lycosa</i>	<i>baulnyi</i>	Guercif	63	34.248 N 3.265 W	KC550799	KC550929	KC551169	
LC1399_MA	<i>Lycosa</i>	<i>baulnyi</i>	Gouttitr	62	34.386 N 3.013 W	KC550801	KC550927	KC551172	KC550890
LC1597_MA	<i>Lycosa</i>	<i>baulnyi</i>	Near Fort Bou-Jerif	90	29.011 N 10.345 W	KC550793		KC551151	
LC1600_MA	<i>Lycosa</i>	<i>baulnyi</i>	Near Fort Bou-Jerif	90	29.011 N 10.345 W	KC550794	KC551038		KC550908
LC1606_MA	<i>Lycosa</i>	<i>baulnyi</i>	IknioulN (Jebel Sahro)	77	31.171 N 5.714 W			KC551152	KC550910
LCX6M9_MA	<i>Lycosa</i>	<i>baulnyi</i>	Tagalmoust	86	29.807 N 9.53 W	KC550803	KC550936	KC551162	
LC1001_TN	<i>Lycosa</i>	<i>munieri</i>	Hdadra	50	35.62 N 10.6 E	KC550749	KC550945	KC551134	KC550830
LC1003_TN	<i>Lycosa</i>	<i>munieri</i>	Ayn Drahim	42	36.737 N 8.679 E	KC550750	KC551005	KC551117	KC550832
LC1041_TN	<i>Lycosa</i>	<i>munieri</i>	Tajeourine	47	35.859 N 8.494 E	KC550753	KC551051	KC551120	KC550835
LC1056_TN	<i>Lycosa</i>	<i>munieri</i>	Manzil Salim	36	37.114 N 9.674 E	KC550751	KC550986	KC551118	KC550836
LC1070_TN	<i>Lycosa</i>	<i>munieri</i>	Mateur, Ichkeul Lake	35	37.114 N 9.674 E	KC550752	KC551019	KC551130	KC550840
LC1086_TN	<i>Lycosa</i>	<i>munieri</i>	Qalat-As-Sanan	49	35.751 N 8.362 E	KC550767	KC550976		KC550841
LC1297_SA	<i>Lycosa</i>	<i>munieri</i>	Sassari	11	40.709 N 8.433 E	KC550769	KC551056	KC551137	KC550868
LC1319_SA	<i>Lycosa</i>	<i>munieri</i>	Dorgali	14	40.304 N 9.591 E		KC551006	KC551135	KC550871
LC1322_SA	<i>Lycosa</i>	<i>munieri</i>	Galtellí	13	40.33 N 9.617 E	KC550755	KC550952	KC551136	KC550872
LC1326_SA	<i>Lycosa</i>	<i>munieri</i>	Igrèsias	31	39.238 N 8.556 E	KC550757		KC551138	
LC1332_SA	<i>Lycosa</i>	<i>munieri</i>	Igrèsias	31	39.238 N 8.556 E	KC550756	KC550963	KC551139	KC550873
LC1334_SA	<i>Lycosa</i>	<i>munieri</i>	Perdasdefogu	25	39.659 N 9.516 E	KC550754	KC551042	KC551140	KC550874
LC1340_IB	<i>Lycosa</i>	<i>munieri</i>	Porto Pedro, Santanyí	29	39.377 N 3.196 E	KC550758	KC551080	KC551119	KC550875
LC1506_IB	<i>Lycosa</i>	<i>munieri</i>	Son Granada de Baix	28	39.458 N 2.768 E	KC550763	KC550971	KC551126	
LC1507_IB	<i>Lycosa</i>	<i>munieri</i>	Son Granada de Baix	28	39.458 N 2.768 E	KC550759	KC551004	KC551123	KC550903
LC1524_IB	<i>Lycosa</i>	<i>munieri</i>	Ses Salines	30	39.33 N 3.079 E	KC550762	KC551037	KC551128	KC550904
LC1555_IB	<i>Lycosa</i>	<i>munieri</i>	Coll de Cucons	27	39.611 N 3.217 E	KC550764	KC551060	KC551127	KC550905
LC1560_IB	<i>Lycosa</i>	<i>munieri</i>	Fornalutx	21	39.784 N 2.734 E	KC550765	KC551007	KC551124	KC550906
LC1561_IB	<i>Lycosa</i>	<i>munieri</i>	Fornalutx	21	39.784 N 2.734 E	KC550766	KC551030	KC551129	
LC1572_IB	<i>Lycosa</i>	<i>munieri</i>	Camí Vell de Campanet	19	39.817 N 2.991 E	KC550760	KC551082	KC551122	KC550907
LC1575_IB	<i>Lycosa</i>	<i>munieri</i>	Camí Vel de Campanet	19	39.817 N 2.991 E	KC550761	KC550972	KC551125	
LCMA01_IB	<i>Lycosa</i>	<i>munieri</i>	Mallorca	92	Unknown	KC550770	KC550957	KC551131	KC550917
LCMA10_IB	<i>Lycosa</i>	<i>munieri</i>	Mallorca	92	Unknown	KC550772	KC551054	KC551132	
LCMA11_IB	<i>Lycosa</i>	<i>munieri</i>	Mallorca	92	Unknown	KC550771	KC551071	KC551133	
LCMENO_IB	<i>Lycosa</i>	<i>munieri</i>	Torret de Sant Lluís	18	39.835 N 4.25 E	KC550768	KC551048	KC551121	KC550918
AL1187_PI	<i>Lycosa</i>	<i>fasciventris</i>	Rodalquilar	40	36.847 N 2.043 W	KC550720	KC551075	KC551094	

Table 1 (continued)

Code <sup>a</sup>	Genus	Species	Locality	L. N. <sup>b</sup>	Lat_Long	COI	12S	16S	28S
AL1189_PI	<i>Lycosa</i>	<i>fasciiventris</i>	La Boca de los Frailes	41	36.806 N 2.133 W	KC550728	KC551052	KC551099	
AL1201_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Rodalquilar	40	36.847 N 2.043 W	KC550736	KC551064		
AL1209_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Rodalquilar	40	36.847 N 2.043 W	KC550734	KC551017	KC551095	
AL9907_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Rodalquilar	40	36.847 N 2.043 W	KC550721	KC550980	KC551096	
AL9908_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Rodalquilar	40	36.847 N 2.043 W	KC550722	KC551079	KC551101	KC550820
AL9909_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Rodalquilar	40	36.847 N 2.043 W	KC550729	KC550965	KC551097	
AL9910_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Rodalquilar	40	36.847 N 2.043 W	KC550735	KC551086	KC551098	
AL9911_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Rodalquilar	40	36.847 N 2.043 W	KC550724	KC550992	KC551100	
CA1138_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Los Barrios	45	36.221 N 5.558 W	KC550738	KC551045		KC550822
GE1178_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Madrid	16	40.289 N 3.685 W	KC550723	KC551083		KC550828
LC1113_MA	<i>Lycosa</i>	<i>fasciiventris</i>	Oued Arozan, Beni Merissa	53	35.383 N 5.338 W	KC550740	KC551027	KC551110	KC550844
LC1132_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Sagunt	26	39.638 N 0.234 W		KC551014		KC550846
LC1133_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Sagunt	22	39.72 N 0.342 W	KC550812	KC550967		KC550847
LC1134_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Sagunt	23	39.701 N 0.334 W	KC550732	KC550973		
LC1139_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Sagunt	24	39.7 N 0.275 W	KC550813	KC551040		
LC1168_MA	<i>Lycosa</i>	<i>fasciiventris</i>	Near Ouzoud Falls, Azilal	74	32.195 N 6.54 W	KC550746	KC550999		KC550852
LC1225_MA	<i>Lycosa</i>	<i>fasciiventris</i>	Near Douar Abd el Jelil	64	34.171 N 4.834 W	KC550741	KC550988	KC551114	KC550863
LC1226_MA	<i>Lycosa</i>	<i>fasciiventris</i>	Stehat	55	35.34 N 4.962 W	KC550745	KC550974	KC551112	KC550864
LC1227_MA	<i>Lycosa</i>	<i>fasciiventris</i>	Cap Spartel, Tanger	48	35.785 N 5.903 W	KC550737	KC551011	KC551116	
LC1246_MA	<i>Lycosa</i>	<i>fasciiventris</i>	Xauen	56	35.186 N 5.285 W	KC550748	KC550958	KC551115	KC550865
LC1250_MA	<i>Lycosa</i>	<i>fasciiventris</i>	Umeras	54	35.362 N 5.373 W	KC550747	KC551039	KC551111	KC550866
LC1252_MA	<i>Lycosa</i>	<i>fasciiventris</i>	El Fendek, Tetuan	51	35.552 N 5.583 W	KC550739	KC551033	KC551108	KC550867
LC1289_MA	<i>Lycosa</i>	<i>fasciiventris</i>	Cap Spartel, Tanger	48	35.785 N 5.903 W	KC550742	KC551066	KC551109	
LC1343_PI	<i>Lycosa</i>	<i>fasciiventris</i>	Nijar	38	36.98 N 2.171 W	KC550730	KC550982	KC551107	KC550876
LC1367_MA	<i>Lycosa</i>	<i>fasciiventris</i>	Azoura	84	30.025 N 8.59 W	KC550743	KC550924		KC550882
LC1378_MA	<i>Lycosa</i>	<i>fasciiventris</i>	Zaouia ech Cheïkh	73	32.623 N 5.974 W	KC550744	KC550931	KC551113	KC550885
LC1480_FR	<i>Lycosa</i>	<i>fasciiventris</i>	Caves, Aude	5	42.935 N 2.968 E	KC550725	KC550984	KC551102	
LC1485_FR	<i>Lycosa</i>	<i>fasciiventris</i>	Caves, Aude	5	42.935 N 2.968 E	KC550726	KC551062	KC551103	KC550900
LC1490_FR	<i>Lycosa</i>	<i>fasciiventris</i>	L'Hospitalet, Herault	3	43.161 N 3.142 E	KC550727	KC550996	KC551104	KC550901
LC1493_FR	<i>Lycosa</i>	<i>fasciiventris</i>	L'Hospitalet, Herault	3	43.161 N 3.142 E	KC550731	KC551058	KC551105	
LC1497_FR	<i>Lycosa</i>	<i>fasciiventris</i>	L'Hospitalet, Herault	6	43.17 N 3.107 E	KC550733	KC550961	KC551106	
LC1468_PI	<i>Pisaura</i>	<i>mirabilis</i>	Torla		42.64 N 0.11 W	KC550814		KC551233	KC550898
LC1440_MA	<i>Arctosa</i>	<i>alluaudi</i>	Fôret Maamora	65	34.092 N 6.202 W	KC550815	KC550938	KC551232	KC550897
CA1144_PI	<i>Hogna</i>	<i>radiata</i>	Los Barrios	45	36.221 N 5.558 W	KC550817	KC551070	KC551092	KC550823
LC1315_SA	<i>Hogna</i>	<i>radiata</i>	Laerru	8	40.823 N 8.871 E	KC550816	KC551016	KC551093	KC550870

<sup>a</sup> Geographic region abbreviations: FR (France), IB (Balearic Islands), IT (Italian Peninsula), MA (Morocco), PI (Iberian Peninsula), SA (Sardinia), TN (Tunisia).

<sup>b</sup> Locality number as in Fig. 1.

Table 2

List of primers used in this study.

Gene fragment	Primer	Or. <sup>a</sup>	Sequence (5'–3')	Reference
<i>cox1</i>	LCOI	F	GGTCAACAATCATAAAGATATTGG	Folmer et al. (1994)
	HCOI	R	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
	MT6	F	GGAGGATTGGAAATTGATTAGTTCC	Simon et al. (1994)
	NANCY	R	CCCGGTAATAAATAAATAAATTC	Simon et al. (1994)
	JERRY	F	CAACATTTATTTGATTTTTTGG	Simon et al. (1994)
	MAGGIE	R	GGATAATCAGAATATCGTCGAGG	Hedin and Maddison (2001)
<i>rrnS</i>	12St-L	F	GGTGGCATTITTTATTTATTAGAGG	Croom et al. (1991)
	12Sbi-H	R	AAGAGCGACGGCGATGTGT	Simon et al. (1994)
<i>rrnL</i> + tRNA-Leu + nad1	16SF	F	CGCCCTGTTTAACAAAAACAT	Kocher et al. (1989)
	16SR	R	CCITTAACGAATTTGAATATA	Hedin and Maddison (2001)
	New16SRLyc	R	GGCAGAAATAATGCATTAGAATTAG	This study
SSU	Lyc28SFa	F	GGAAGTAAGAGTAGGGCTTCCC	This study
	Lyc28SRa	R	ATGGTTCGATTAGTCTTTCGCCCC	This study

<sup>a</sup> Primer orientation.

default values, except for the transition/transversion rates, which were changed to a uniform distribution with 0–100 based on preliminary analyses. Convergence was assessed with EES parameters in Tracer v. 1.4 (Rambaut and Drummond, 2007). A 10% burn-in of each analysis and tree combination was carried out in LogCombiner and TreeAnnotator (Drummond and Rambaut, 2007). Both the single threshold (Pons et al., 2006) and multiple threshold (Monaghan et al., 2009) models were applied to the tree, and a Likelihood Ratio Test (LRT) was applied to compare the models.

## 2.5. Phylogenetic analyses

Bayesian Inference (BI) was used to investigate optimal tree topology using the program MrBayes v. 3.1.2 (Ronquist and Huel- senbeck, 2003). For each analysis, four Markov chains were run (one cold, three heated) for 5 million iterations, saving trees at each 1000th generation. This procedure was performed twice to check for convergence. The average standard deviation of the split frequencies of the two runs (ASDSF < 0.01) was used to ensure that

**Table 3**  
Dataset information.

	Gene fragment	Align. length <sup>a</sup>	Var <sup>b</sup>	Model <sup>c</sup>
Species delimitation dataset	<i>cox1</i>	1196	278	GTR + G
	<i>nadh</i>	88	34	HKY + I
	12S	259	67	GTR + G
	16S + tRNA-Leu	586	91	GTR + G
Phylogenetic analyses dataset	<i>cox1</i>	1196	336	GTR + G
	<i>nadh</i>	88	40	HKY + I + G
	12S	259	78	GTR + G
	16S + tRNA-Leu	593	164	GTR + I + G
	28S	612	115	GTR + G

<sup>a</sup> Alignment length.<sup>b</sup> Variable sites.<sup>c</sup> Appropriate models selected by jModeltest.

the Markov chains had reached stationarity. The burn-in performed discarded the first 25% trees of each run, and a majority-rule consensus tree was generated from the remaining trees. Independent analyses were carried out for each partition and for the combined dataset. In the combined dataset, the best fitting model was applied to each partition, and all parameters were unlinked across partitions.

Maximum Likelihood (ML) analyses were conducted using RAxML 7.0.3 (Stamatakis, 2006). The evolutionary model and its parameters were estimated independently for each of the partitions and a non-parametric bootstrap support analysis of 1000 pseudoreplicates was conducted.

## 2.6. Estimation of divergence times

Dating analyses were conducted using BEAST v. 1.6.2 (Drummond and Rambaut, 2007). For species-level phylogenies, coalescent priors are generally inappropriate, and thus, a Yule (pure birth) model prior was used in a dataset containing only one representative per GMYC cluster. Using a *Lycosa*-specific rate would be the most recommendable approximation for the dating analyses, but within the Western Mediterranean *Lycosa* there is no reliable calibration point and, thus, we applied a spider-specific molecular evolution rate to each gene partition (Bidegaray-Batista and Arnedo, 2011) as it has been shown to be preferable to applying the “standard mitochondrial rate of 2.3%” (Andujar et al., 2012) as a global rate. We conducted analyses using a relaxed uncorrelated lognormal molecular clock and an unlinked evolutionary model selected from jModeltest applied at each mitochondrial partition during 30 million generations. Two independent runs were conducted, and after assessing convergence by checking the ESS parameters in Tracer v. 1.4 (Rambaut and Drummond, 2007), the runs were merged, applying a 10% burn-in using Logcombiner and TreeAnnotator (Drummond and Rambaut, 2007).

## 3. Results

### 3.1. Species delimitation

The data matrix used in the species delimitation analyses included 180 terminals and 2129 aligned characters (see Table 3 for dataset information).

A comparison of the performance between the single and multiple threshold GMYC models revealed that these methods were not significantly different from each other ( $X^2 = 1.3033$ ,  $df = 6$ ,  $p = 0.9715$ ). Furthermore, both models provided a significantly better fit to the data than the null model (Single method;  $p = 0.0006$ , Multiple method;  $p = 0.0024$ ); consequently, we used the results of the single threshold model for further analysis. In this case, 12

clusters were detected (CI: 10–14) (Fig. 2), and the delimited GMYC clusters were completely congruent with the monophyletic and well-supported clades obtained using the Bayesian Inference analyses.

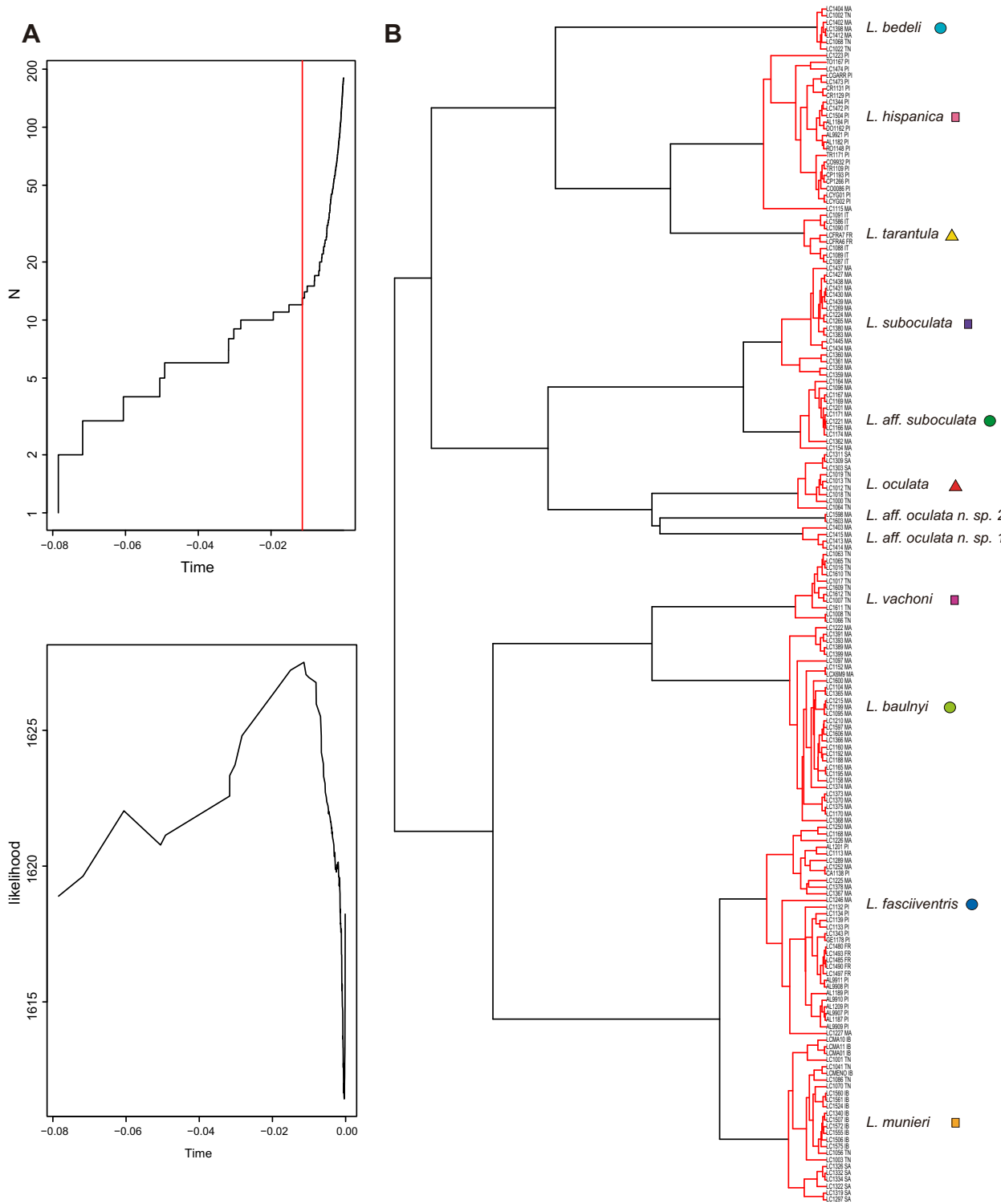
### 3.2. Molecular phylogeny

The dataset used for the phylogenetic analyses included one representative per sampled locality and GMYC cluster and three outgroups, ending with 107 terminal taxa and 2748 aligned characters (see Table 3 for dataset information, Fig. S1 in the Supplementary material and TreeBASE ID:13887).

Independent BI analyses of individual genes produced trees with topological differences (results not shown), but the conflicting nodes had low values of posterior probability (pp) support (<0.9) (see Fig. 2), mainly because of the lack of phylogenetic information. It was therefore concluded that there were no major topological conflicts to impede concatenation.

The topology produced by the ML analysis using the concatenated dataset (phylogenetic analyses dataset, Table 3) was very similar to that obtained with BI analysis, with the main differences occurring in the *L. munieri* Simon, 1876 and *L. fasciiventris* Dufour, 1835 clades. ML analysis did not recover the monophyly of *L. munieri*, leaving it paraphyletic with respect to *L. fasciiventris*. Both methods recovered four major monophyletic groups (see Fig. 3): (1) the *Lycosa tarantula* group, (2) the *Lycosa oculata* group, (3) the *Lycosa baulnyi* group and (4) the *Lycosa fasciiventris* group. All four groups are well supported in both analyses (Bayesian posterior probability (pp) > 0.99 and ML bootstrap support > 96) and constitute two main evolutionary lineages: *L. tarantula* group–*L. oculata* group (pp = 0.94, ML bootstrap = 85) and *L. baulnyi* group–*L. fasciiventris* group (pp = 0.91, ML bootstrap = 80).

In the *L. tarantula* group, the Maghrebian *L. bedeli* Simon, 1876 is a sister species to the European *L. tarantula* (Linnaeus, 1758) and *L. hispanica* (*L. tarantula* includes specimens from France that were identified as *L. narbonensis* Walckenaer, 1806 but are genetically close to *L. tarantula* specimens from Italy). In the *L. oculata* group, five different well-supported lineages, delimited as GMYC clusters and thus putative species, were found grouped into two reciprocally monophyletic groups. In the former, *L. aff. oculata1* is sister to *L. oculata* Simon, 1876 and *L. aff. oculata2*, while the latter is composed of two widely distributed species from the western part of Morocco, *L. suboculata* Guy, 1966 and *L. aff. suboculata*. The *Lycosa baulnyi* group includes two well-supported North African species, *L. baulnyi* and *L. vachoni* Guy, 1966, and the *Lycosa fasciiventris* group consists of two widely distributed species, *L. munieri* and *L. fasciiventris*.



**Fig. 2.** (A) Lineage through time (above) and likelihood surface plots (below) from the Generalized mixed Yule-coalescent species delimitation analysis. The red line indicates the shift from interspecies (phylogenetic Yule processes) to intraspecies branching events (coalescent population processes). Time is scaled from  $-1$  to  $0$ . (B) GMYC tree. GMYC clusters delimited from the single-threshold model are highlighted in red and labeled according to their defined species. Symbols next to each species name correspond to Fig. 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Divergence dating

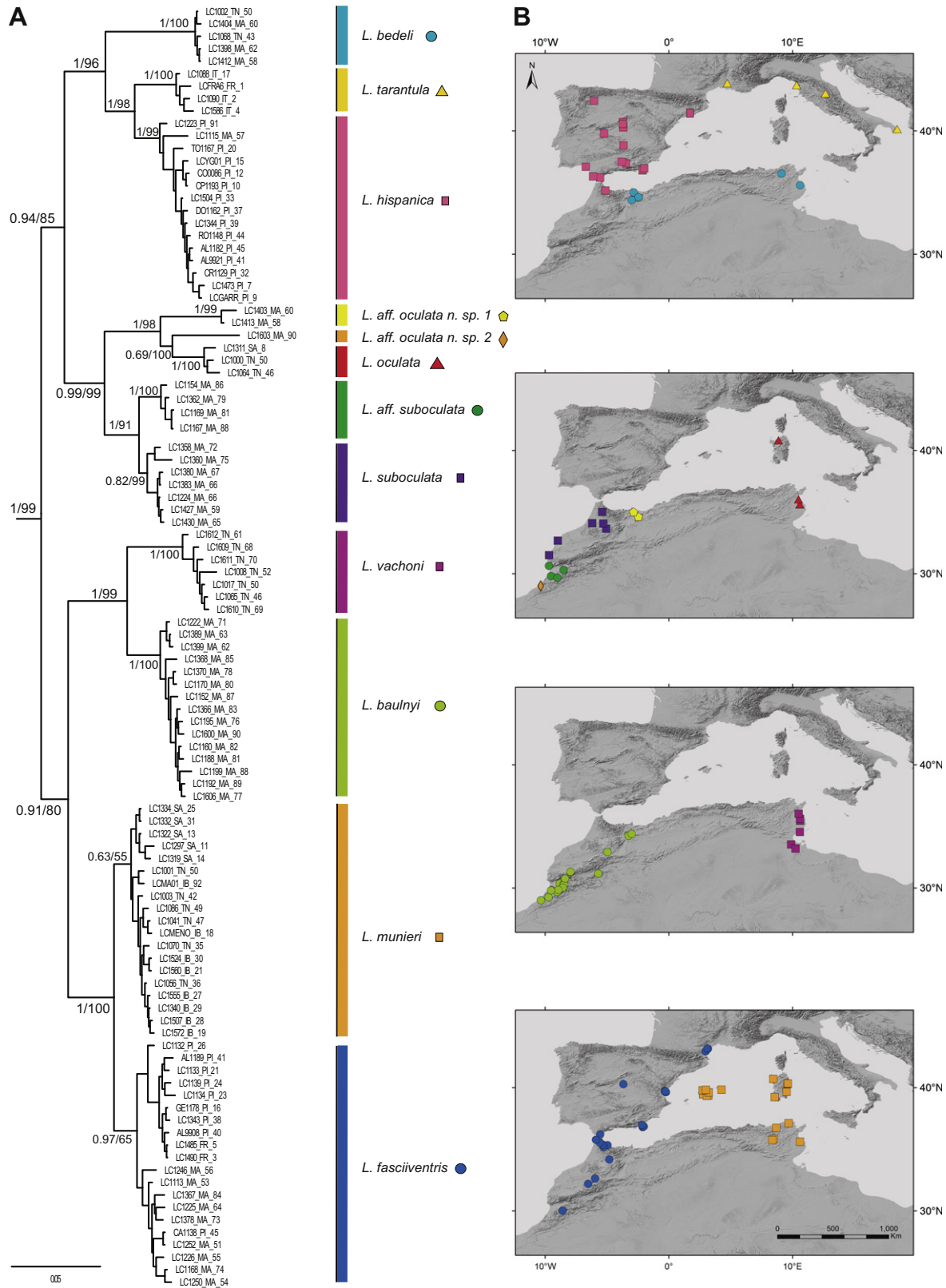
The cladogram obtained in BEAST is shown in Fig. 4. The Western Mediterranean *Lycosa* is estimated to have originated in the early Pliocene, and the majority of speciation events appear to have occurred during the Pleistocene.

4. Discussion

4.1. Species delimitation and taxonomic implications

The current study presents the first comprehensive hypothesis for the phylogenetic relationships among wolf spiders in the genus





**Fig. 3.** (A) BI tree of the genus *Lycosa* inferred using the concatenated dataset of *cox1*, 16S, tRNA-Leu, *nadh* and 12S mtDNA gene fragments and 28S nuDNA referred to as combined dataset in the text. Numbers next to nodes correspond to posterior probability values in the BI analysis and to bootstrap support in the ML analysis respectively. The tree was rooted using *Pisaura mirabilis* Outgroups are not shown. Next to each specimen code, geographic region and locality are provided as shown in Table 1. (B) Distributional maps of each *Lycosa* species group.

*Lycosa* in the Western Mediterranean, including *Lycosa tarantula*, the type species of the genus, and thus establishes the framework to set a proper generic diagnosis. We succeeded in obtaining repre-

sentatives of all western European and nine Maghrebian species. However, we should also note that it is likely that more species exist, especially in undersampled areas, such as Algeria, where a large

number of species have been described, but also in well-sampled areas due to the temporal succession of occurring species (Parella-da, 1998), leaving uncertainty in the real number of extant species.

All clusters recovered using the GMYC analyses as species delimitation method correspond to monophyletic evolutionary lineages with allopatric distributions and congruent sexual character morphologies. We obtained a robust phylogeny of the genus *Lycosa* from the Western Mediterranean (Fig. 3) with both Maximum Likelihood and Bayesian Inference reconstruction methods. The inferred molecular phylogeny clearly shows that all Western Mediterranean *Lycosa* species included in the analysis form a monophyletic group (Fig. S1 in the Supplementary material, TreeBASE ID: 13887). These results indicate that all these species belong to the genus *Lycosa*, highlighting the degree of confusion surrounding this group of spiders. Six out of the 12 species included in the analysis were previously regarded as belonging to the genera *Allocosa* and *Hogna*.

#### 4.1.1. The *Lycosa tarantula* group

This clade is formed by three relatively large wolf spider species that construct permanent burrows with the entrance surrounded by a conspicuous turret made of leaves, twigs and other debris and fastened together with silk that provides protection against predators (Williams et al., 2006). These species are typically found in semiarid Mediterranean areas and are distributed throughout the Western Mediterranean. This group includes *L. tarantula*, the type species of the genus, as well as *L. bedeli* and *L. hispanica*. The Maghrebian *L. bedeli* is the sister species of the two European representatives (*L. tarantula* and *L. hispanica*). *Lycosa bedeli* was described by Simon in 1876 from the Algerian locality of Géryville, currently known as El Bayadh. This species is considered to be distributed from Morocco to Tunisia (Guy, 1966). In our analyses, we included individuals from the Beni Snassen massif in the eastern part of Morocco and from two localities in Tunisia. The small intra-specific genetic *p*-distance found between the Moroccan and Tunisian populations (<0.2% for *cox1*) suggests that this species could be common and widely distributed throughout Algeria.

*Lycosa tarantula*, originally named *Aranea tarantula*, is the 28th of the 35 species described by Linnaeus in his *Systema Naturae* (1758). In our analyses, we included individuals from Otranto, in the Italian region of the Puglia, which is considered the species type locality, and individuals from different regions of the Italian Peninsula. The results clearly show that the species forms a monophyletic clade, and it appears as an independent cluster in the GMYC analysis. This group also includes individuals from France identified as *L. narbonensis* that are not genetically distinct from the Italian representatives (Figs. 2 and 3). Walckenaer (1806) described *L. narbonensis* as a variety of *L. tarantula*, and he did not provide a specific locality but rather described a large distribution range. This distribution was circumscribed in a subsequent re-description by Simon (1876), indicating that it is typically from “le Midi de la France” (the southern part of France) and the north of Italy, and the species was named due to its commonality in the environments of Narbonne (Simon, 1864). The morphological differences between *L. tarantula* and *L. narbonensis* have never been clearly discussed, and its distinction as separate species is mainly based on its supposed allopatry. According to our results, we consider *L. narbonensis* as a junior synonym of *L. tarantula*, given the lack of genetic and morphological distinctiveness between specimens from these populations.

The sister species of *L. tarantula*, *L. hispanica*, is found on the other side of the Pyrenees. This Iberian species forms a well-supported monophyletic clade that constitutes an independent cluster in GMYC analyses. The species was described by Walckenaer in 1837 but was previously characterized by Dufour (1835) (under the name *L. tarantula*) in an excellent description of their biology. After Rower’s revision (1955), this species was transferred to the genus *Hogna*, and its taxonomic status has not been revised since. Having checked the material identified by Simon at the MNHN of Paris, and given the original description, we propose transferring the species back to the genus *Lycosa*, recovering the original combination *Lycosa hispanica* Walckenaer, 1837. Although *L. hispanica* is typically found on the Iberian Peninsula, we also found one representative of the species in the Rif Mountains (Morocco), implying

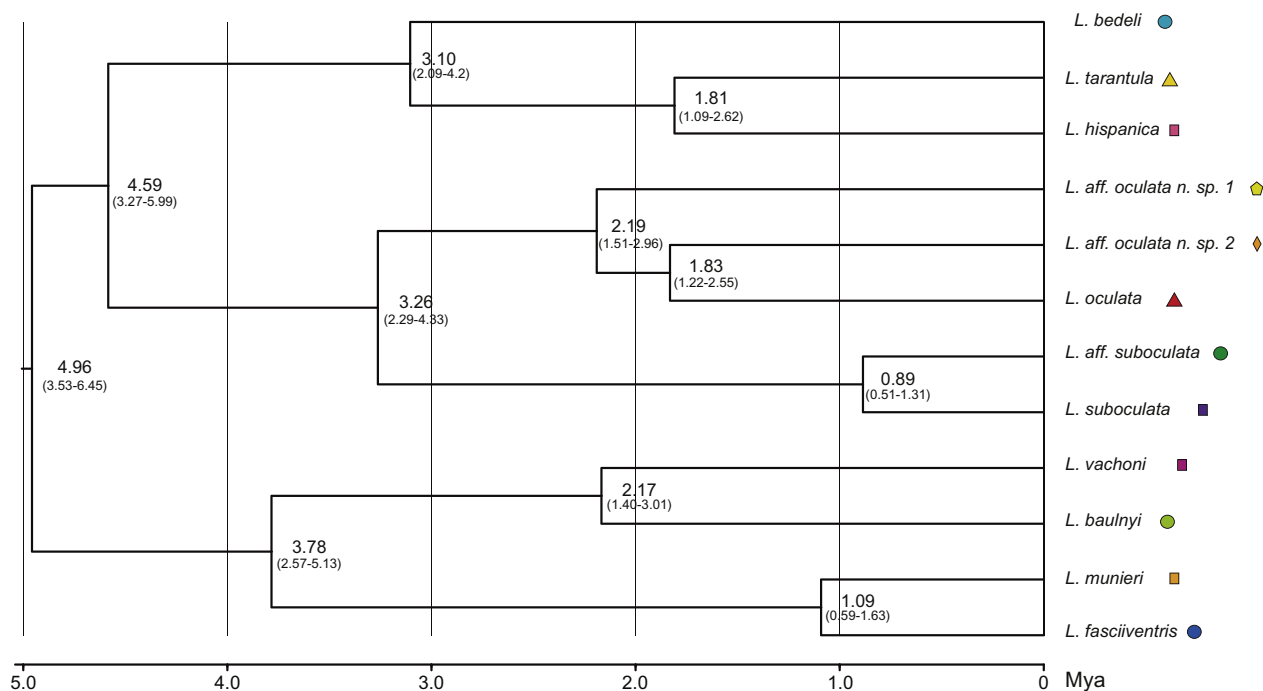


Fig. 4. mtDNA chronogram tree representing the divergence time estimations and, in parentheses, the relative 95% confidence intervals for each dichotomy. Numbers are expressed in million years (Mya).

a recent colonization process from the Iberian Peninsula to Morocco. This could be explained if we take into account the propensity of the juvenile wolf spiders for ballooning (Murphy et al., 2006, and references therein). This pattern has also been found in other groups for which the Gibraltar Strait does not represent an impenetrable biogeographic barrier and for which colonization events have been dated long after the last land connection, that occurred 5.3 Mya during the Messinian Salinity Crisis (Carranza et al., 2006; Cosson et al., 2005; Gaubert et al., 2010; Harris et al., 2002).

Our results indicate that the ancestor of the current European representatives of the group dispersed via one of the two possible colonization routes; Morocco–Strait of Gibraltar–Iberian Peninsula or Tunisia–Sicily–Italian Peninsula, after separating from the ancestor of the North African *L. bedeli* (3.10 Mya, 95% HDP: 2.09–4.2 Mya). This colonization process took place long after the aforementioned Messinian Salinity Crisis (5.3 Mya). The two allopatric European species, *L. tarantula* and *L. hispanica*, diverged approximately 1.8 Mya (1.09–2.62 Mya) (Fig. 4) during the late Pliocene. In this scenario, and taking into account the current distribution of both European species, the speciation process could have been triggered by the onset of the Pliocene glacial cycles and the subsequent use of the two peninsulas as southern glacial refuge, a pattern widely reported among European fauna (Hewitt, 2000; Schmitt, 2007; Stewart et al., 2010; Taberlet et al., 1998).

#### 4.1.2. The *Lycosa oculata* group

Five putative species within this group were found in our analyses. All of these species construct burrows with trapdoors made of sand and silk, which can be very well camouflaged. In 1885, Simon described this peculiar burrowing behavior in his description of *Lycosa cunicularia* (= *L. oculata*) from Tunisia (Simon, 1885). He noted the similarity of the burrow trapdoor with those built by species of the genus *Nemesia* (Mygalomorphae, Nemesiidae). *Lycosa cunicularia* was later synonymised with *L. oculata*, which was described from Corsica by the same author. Although *L. oculata* was not known to be present on Sardinia, we recovered a monophyletic group composed by individuals from Sardinia and Tunisia that are morphologically indistinguishable from the *L. oculata* illustrations from Corsica and the specimens deposited in the MNHN in Paris. Thus, our results corroborate the placement of *L. cunicularia* as a junior synonym of *L. oculata*.

In the same clade with *L. oculata*, we recovered two additional putative species that constitute independent clusters in GMYC analyses: *L. aff. oculata* n. sp. 1 and *L. aff. oculata* n. sp. 2. Our results indicate that *L. aff. oculata* n. sp. 2 is sister species to *L. oculata*, with a genetic *p*-distance divergence of 4.5%. *Lycosa aff. oculata* n. sp. 1 is sister to the former group with a genetic *p*-distance of 4.4% with respect to both species. Unfortunately, we could not obtain adult specimens of *L. aff. oculata* n. sp. 1 or *L. aff. oculata* n. sp. 2, and it is therefore necessary to increase sampling efforts to collect adult material and confirm their identity by morphological studies. *Lycosa aff. oculata* n. sp. 1 was sampled from two adjacent areas of the Beni Snassen massif in northeastern Morocco, and *L. aff. oculata* n. sp. 2 was collected in the southwestern part of Morocco near the mouth of Oued Assaka in a semidesert area. It is surprising that *L. aff. oculata* n. sp. 2 is more closely related to *L. oculata* than to *L. aff. oculata* 1 even though it is geographically more separated from *L. oculata*. Moreover, this relationship should be interpreted with caution because it is plausible that more species from this group are distributed in Algeria; further studies are necessary.

The two remaining species, *L. suboculata* and *L. aff. suboculata*, present 2.2% (*cox1*) of genetic divergence between them (Table 4) and appear as monophyletic entities in GMYC analysis. This clade splits from the above clade at 3.26 Mya (2.29–4.33 Mya) (Fig. 4). Additionally, clear morphological differences in the epigyne and the vulva of these species confirm their distinctiveness. *Lycosa sub-*

*oculata* is distributed from the northern slopes of the Rif Mountains to the Atlas Mountains through the Atlantic coast of Morocco, while *L. aff. suboculata* is found in the southwestern Sous Valley and adjacent Anti-Atlas Mountains. The recent segregation between these species at 0.89 Mya (0.51–1.31 Mya) and their distributions suggest that climatic oscillations due to recent glacial cycles promoted this allopatric speciation, also found in other groups (Husemann et al., 2012).

The description of *L. suboculata* was based on two juvenile females (see drawings from original description in Guy (1966)) without any locality provided. In the same study, Guy noted the presence of this species near Asni, Morocco. Juvenile females from both species are similar to the drawings from the original description. Although we were unable to collect specimens from Asni, which is the only known locality for *L. suboculata*, our localities are also in the western part of the Atlas Mountains, and thus we provisionally consider the specimens reported here to belong to *L. suboculata*.

After Roewer's (1955) revision, *L. oculata* was transferred to the genus *Allocosa*. Later, Brignoli (1983) transferred *L. suboculata* to the same genus. Our results indicate that both species belong to the genus *Lycosa*, and, therefore, we propose that they should be transferred back to this genus. Further collecting trips should be performed to obtain adult specimens of the species mentioned above before proceeding to their description or redescription.

#### 4.1.3. The *Lycosa baulnyi* group

In the studied area, this group is formed by two species, *L. baulnyi* and *L. vachoni*, that are found exclusively in the Maghreb (Fig. 3). They construct burrows with a turret surrounding the entrance, although the burrows are not as deep as those of the *L. tarantula* group. *Lycosa baulnyi* was first described by Simon (1876) based on female specimens from Bou Saâda (Algeria); later, Strand (1908) described the male from Saïda (Algeria). *Lycosa baulnyi* was transferred to the genus *Allocosa* by Roewer (1955), and here we propose to transfer it back to the genus *Lycosa*. This species has been recorded in Morocco, Algeria and Tunisia (Guy, 1966). In this study, we included specimens from 16 localities in Morocco, ranging from the southwestern region near Guelmin to the northeastern region near Taourirt. Despite intense efforts, we did not find *L. baulnyi* in Tunisia, and the published records of *L. baulnyi* from this country all date to before 1966, when *Lycosa vachoni*, the second species of this group was described. Thus, the reports of *L. baulnyi* in Tunisia must be considered questionable due to the morphological similarity of the external genitalia of these two species. *Lycosa vachoni* was described by Guy (1966) based on female specimens from Sélîm (Algeria), which have been deposited in the MNHN, while the male is still unknown. Guy (1966) noted differences in the epigyne, which are more conspicuous in the vulva, that we have been able to confirm. In this study, we included *L. vachoni* specimens from seven localities in eastern Tunisia.

In our analyses, *L. vachoni* and *L. baulnyi* were always recovered as two monophyletic lineages, as independent clusters in the species delimitation analyses, and with relatively high genetic divergence (4.6% uncorrected *p*-distances of *cox1*) (Table 4). The split between *L. vachoni* and *L. baulnyi* has been dated at 2.17 Mya (1.4–3.01 Mya) (Fig. 4). The lack of specimens from Algeria, where the two species are reported to cohabit, prevents a better clarification of the evolutionary history of the group. More samples, especially from the contact zone, would be expected to shed light on the causes of speciation.

#### 4.1.4. The *Lycosa fasciventris* group

This group is composed of two closely related species with a genetic *p*-distance of 3.3% *cox1*, *Lycosa fasciventris* and *Lycosa munieri*

**Table 4** Mean uncorrected p-distances between and within GMYC clusters. Upper right: values for 12S and 16S; lower left: values for *cox1*, all between species. Diagonal (bold): values within each species (*cox1*/12S/16S).

	<i>L. bedeli</i>	<i>L. tarantula</i>	<i>L. hispanica</i>	<i>L. aff. oculata</i> n. sp. 1	<i>L. aff. oculata</i> n. sp. 2	<i>L. oculata</i> n. sp. 2	<i>L. suboculata</i>	<i>L. aff. suboculata</i>	<i>L. vachoni</i>	<i>L. baulnyi</i>	<i>L. munieri</i>	<i>L. fasciventris</i>
<i>L. bedeli</i>	<b>0.002/0/0.001</b>	0.047/0.028	0.032/0.030	0.081/0.054	0.081/0.053	0.069/0.048	0.070/0.035	0.069/0.035	0.066/0.044	0.066/0.040	0.061/0.028	0.058/0.031
<i>L. tarantula</i>	0.063	<b>0.007/0.010/0.002</b>	0.028/0.018	0.063/0.052	0.074/0.047	0.059/0.044	0.066/0.031	0.061/0.031	0.052/0.044	0.061/0.040	0.061/0.033	0.058/0.036
<i>L. hispanica</i>	0.062	0.034	<b>0.008/0.009/0.007</b>	0.068/0.050	0.058/0.049	0.058/0.049	0.067/0.033	0.064/0.030	0.059/0.045	0.065/0.038	0.058/0.031	0.056/0.033
<i>L. aff. oculata</i> n. sp. 1	0.070	0.063	0.068	<b>0.009/0.005/0.006</b>	0.047/0.042	0.027/0.030	0.041/0.043	0.049/0.043	0.078/0.057	0.072/0.057	0.070/0.044	0.066/0.044
<i>L. aff. oculata</i> n. sp. 2	0.076	0.079	0.076	0.044	n/n/n	0.030/0.030	0.045/0.044	0.049/0.040	0.086/0.050	0.097/0.047	0.078/0.040	0.076/0.042
<i>L. oculata</i>	0.081	0.080	0.077	0.044	0.045	<b>0.011/0.006</b>	0.030/0.036	0.034/0.035	0.069/0.050	0.068/0.052	0.057/0.036	0.056/0.037
<i>L. suboculata</i>	0.068	0.073	0.076	0.063	0.064	0.062	<b>0.006/0.009/0</b>	0.025/0.011	0.066/0.041	0.068/0.043	0.055/0.025	0.052/0.025
<i>L. aff. suboculata</i>	0.069	0.063	0.066	0.058	0.056	0.059	0.022	<b>0.008/0.010/0.001</b>	0.065/0.041	0.063/0.040	0.055/0.025	0.050/0.025
<i>L. vachoni</i>	0.076	0.071	0.069	0.071	0.074	0.072	0.065	0.068	<b>0.006/0.004/0.006</b>	0.026/0.027	0.043/0.030	0.044/0.030
<i>L. baulnyi</i>	0.069	0.069	0.064	0.063	0.071	0.076	0.071	0.055	0.046	<b>0.006/0.007/0.006</b>	0.051/0.024	0.052/0.026
<i>L. munieri</i>	0.072	0.065	0.063	0.068	0.075	0.068	0.060	0.063	0.054	0.050	<b>0.009/0.002/0.002</b>	0.007/0.004
<i>L. fasciventris</i>	0.075	0.066	0.066	0.071	0.075	0.072	0.074	0.069	0.062	0.059	0.033	<b>0.014/0.003/0.00</b>

(Figs. 2 and 3). Although the genus *Lycosa* has been defined as containing large or very large burrowing species (Zyuzin and Logunov, 2000), these two species do not construct permanent burrows, and thus the generic diagnosis should be modified with respect to this character. These species have the widest distribution in the study area, even colonizing some Mediterranean islands. This vagrant life style may facilitate its dispersal capacity.

Although Dufour described *L. fasciventris* in 1835, little attention has been paid to this species in the 175 years since then, perhaps because the author described it in a footnote of a paper concerning the biology of *L. hispanica*. In contrast to the scarcity of morphological information provided in the original description, the author explicitly mentioned that *L. fasciventris* “is a species that lives under stones in the arid mountains of the environments of Murviedro (=Sagunt) (Kingdom of Valencia)” to differentiate it from *L. hispanica*, which builds permanent burrows, and notes that “this species is smaller than the true Tarentula, which it closely resembles”. In this study, we succeeded in collecting several specimens from the type locality of Sagunt (Iberian Peninsula) (Localities 22, 23, 24 and 26, see Fig. 1). We also found representatives of this species as far north as Narbonne (France) and as far south as Azoura (Anti-Atlas). *Lycosa fasciventris* was transferred to the genus *Allocoxa* after Roewer’s (1955) revision, but we consider it appropriate to transfer it back to the genus *Lycosa*. Guy (1966) described *Lycosa panousei* Guy, 1966 based on a female collected in Azilal (Morocco) and described its range as from the Anti-Atlas Mountains to the Middle Atlas through the Marrakesh region and Casablanca. Based on the epigyne illustration provided in the original description and this geographical distribution, we consider *L. panousei* (transferred to genus *Allocoxa* by Brignoli and Merrett (1983)) to be a junior synonym of *L. fasciventris*. Finally, Barrientos (2004) described a new Iberian *Lycosa* species, *Lycosa ambigua* Barrientos, 2004 from Murcia (Spain). Again, the morphology, genetic analyses and distribution of specimens from the type locality confirm this species as a junior synonym of *L. fasciventris*.

The second species of this group, *L. munieri*, also exhibits a wide distribution. We have collected it from Tunisia, Sardinia and the Balearic Islands. Although *Lycosa* specimens from Mallorca have long been identified as *L. subhirsuta*, this species has been recently transferred to the genus *Arctosa* (Bosmans and Van Keer, 2012). *Lycosa munieri* was described by Simon (1876) from Algeria, and its verified distribution was hitherto restricted to this country, although there have also been some dubious claims of its presence in Morocco (Guy, 1966). With the data presented in this study, the known distribution of this species is extended to three Mediterranean Islands (Mallorca, Menorca and Sardinia) and to Tunisia (Fig. 3). We advocate that their range could be extended through Corsica, Sicily and the southern part of the Italian Peninsula, where it may have been historically confused with *L. tarantula*.

From Mallorca, four additional *Lycosa* species have been described by Koch (1882) (*L. conspersa*, *L. fraisei*, *L. insulana* and *L. simplex*). Roewer (1955) transferred these species to *Trochosula*, *Lycorma* and *Pirata*. Recently, in a revision of the species described by Koch from the Balearic Islands, Bosmans and Van Keer (2012) proposed to return them to their original genus in order to facilitate future studies. The aforementioned authors already commented in reference to these species that they “all show the same type of epigyne” and that “the epigyne resembles the epigyne of *Hogna radiata*”. After reviewing the original descriptions and illustrations and based on epigyne morphology, we propose to transfer them to the genus *Hogna*. This genus is in need of a deep taxonomic revision, and thus, assigning a conclusive taxonomic position to these species remains pending and is certainly beyond the scope of the present study.

#### 4.2. The origin, biogeographical patterns and diversification of Western Mediterranean *Lycosa*

Our data support the hypothesis that northwestern Africa has acted as the diversification center of this group (Zyuzin and Logunov, 2000). All species groups have representatives from this area, and all but one species are also present in the Maghreb (Fig. 3B). The only exception is *L. tarantula*, the type species of the genus, which is distributed in the Italian Peninsula and in the Mediterranean-influenced zone of France.

As mentioned above, several authors have noted that the Lycosidae are a family with a relatively recent evolutionary origin (Alderweireldt and Jocqué, 1992; Wunderlich, 2004), or at least that certain groups of lycosids apparently demonstrate recent speciation processes (Vink and Paterson, 2003; Jocqué and Alderweireldt, 2005). Jocqué and Alderweireldt (2005) hypothesize that most of the representatives of this family have co-evolved with the expansion of different types of open habitats with short vegetation and provide evidence for a possible synchronization between the radiation of most lycosid groups with the expansion of grasslands and other open habitats in Africa since the Miocene, a theory that is consistent with our results. The diversification process of the Western Mediterranean *Lycosa* species dates back to the post-Messinian (5 Mya), a relatively recent date given the high diversity found in the group. The first splits in the Western Mediterranean *Lycosa* correspond to those forming the main species groups reported, which roughly correspond to different ecological strategies. The continuous aridification process that began approximately 10 Mya and increased in importance in the Pliocene (around 4 Mya) had major impacts on the Mediterranean vegetation (Thompson, 2005). These changes could have acted as a catalyst for the diversification process of *Lycosa* species, creating new ecological niches that could be filled by different members of the genus. The mtDNA chronogram (Fig. 4) shows that three main diversification events took place almost synchronously within the Western Mediterranean *Lycosa* at 3.1–3.7 Mya. Both the basal diversification in the *L. tarantula* and *L. oculata* groups and the split between *L. baulnyi* and *L. fasciventris* lineages took place during this period. These events coincide in time with the establishment of a Mediterranean-type climate, that is, the onset of a marked seasonality and stable summer droughts, which promoted the replacement of mixed forest by open, xeric, Mediterranean-like communities (Suc, 1984; Thompson, 2005). This could have facilitated northward expansions in the distribution areas of different *Lycosa* species as new open areas were formed.

During the Pliocene and Pleistocene eras, climate changes often occurred very rapidly (Lowe and Walker, 1997), promoting divergence and speciation in southern European refugia (Taberlet et al., 1998; Hewitt, 2011) and also in northwestern Africa (e.g., Cosson et al., 2005; Guiller et al., 2001). Two additional diversification episodes, dated at approximately 2 Mya and 1 Mya, have had severe impacts on the diversity of Mediterranean *Lycosa* species. In this scenario, the onset of glacial cycles and their related climatic oscillations (cold and warm periods) might have been responsible for the simultaneous speciation events in the different *Lycosa* lineages (see Fig. 4). One paradigmatic case is the speciation between *L. hispanica* and *L. tarantula*, each of which is located in one of the two European Mediterranean peninsulas, which could have acted as refugia during the glaciations.

#### 4.3. Taxonomic implications

Our results indicate that the species described by Koch from Mallorca should be transferred to the genus *Hogna* and that six generic transfers by Roewer (1955, 1959), as listed below, should be reversed; i.e., these species should be transferred back to the

genus *Lycosa*. Moreover, we identify three currently accepted species that appear to be synonymous with previously described species. Finally, five *Lycosa* species described by Franganillo (1918, 1920, 1925, 1926) from the Iberian Peninsula, currently considered as valid species in the World Spider Catalog (Platnick, 2013), should be regarded as nomina dubia. As taxonomic conclusions of this study, we propose the following list of nomenclatural changes:

##### Generic transfers:

*Lycosa hispanica* Walckenaer, 1837 status revised, transferred from *Hogna* back to *Lycosa*.

*Lycosa oculata* Simon, 1876 status revised, transferred from *Allocosa* back to *Lycosa*.

*Lycosa suboculata* Guy, 1966 status revised, transferred from *Allocosa* back to *Lycosa*.

*Lycosa baulnyi* Simon, 1876 status revised, transferred from *Allocosa* back to *Lycosa*.

*Lycosa fasciventris* Dufour, 1835 status revised, transferred from *Allocosa* back to *Lycosa*.

*Lycosa munieri* Simon, 1876 status revised, transferred from *Allocosa* back to *Lycosa*.

*Hogna conspersa* (Koch, 1882) status revised, transferred from *Lycosa*.

*Hogna fraisei* (Koch, 1882) status revised, transferred from *Lycosa*.

*Hogna insulana* (Koch, 1882) status revised, transferred from *Lycosa*.

*Hogna simplex* (Koch, 1882) status revised, transferred from *Lycosa*.

##### Synonymies:

*Lycosa narbonensis* Walckenaer, 1806 = *Lycosa tarantula* (Linnaeus, 1758) syn. nov.

*Allocosa panousei* (Guy, 1966) = *Lycosa fasciventris* Dufour, 1835 syn. nov.

*Lycosa ambigua* Barrientos, 2004 = *Lycosa fasciventris* Dufour, 1835 syn. nov.

##### Nomina dubia:

We have not been able to find the type material of the following species described by Franganillo (1918, 1920, 1925, 1926) and we have indications that all this material has been lost. In addition, in the description of these species, the author did not provide diagnostic characters for their identification to species or genus level and none of them has ever been identified or mentioned by any other author since their original descriptions. Therefore, we propose that they should be considered nomina dubia.

*Lycosa leireana* Franganillo, 1918 nomen dubium.

*Lycosa virgulata* Franganillo, 1920 nomen dubium.

*Lycosa granatensis* Franganillo, 1925 nomen dubium.

*Lycosa malacensis* Franganillo, 1926 nomen dubium.

*Lycosa spiniformis* Franganillo, 1926 nomen dubium.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympv.2013.02.006>.

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