Heredity (2004) 93, 416–422

www.nature.com/hdy

Genetic study of the forest pest *Tomicus piniperda* (Col., Scolytinae) in Yunnan province (China) compared to Europe: new insights for the systematics and evolution of the genus *Tomicus*

Y Duan[1,2,4], C Kerdelhué[1,4], H Ye[2] and F Lieutier[1,3]
[1]*INRA, Zoologie forestière, BP 20619 Ardon, F-45166 Olivet cedex, France;* [2]*Life Science College, Yunnan University, Kunming 650091, PR China;* [3]*Université Orléans, Laboratoire Biologie des Ligneux et des Grandes Cultures, BP 6759, F-45067 Orléans cedex 2, France*

The pine shoot beetle *Tomicus piniperda* is present throughout Eurasia. In Europe, it is considered as a secondary pest that rarely causes tree mortality, while heavy damage is observed in Yunnan Province (China) where it exhibits a novel aggregative behaviour during shoot attack. To understand why the ecological characteristics of the European and Chinese populations differ so strongly, we conducted an analysis of population genetic structure on 12 populations in Yunnan and one in JiLin using mitochondrial (COI-COII) and nuclear (ITS2 and 28S rDNA) DNA sequences, and compared the results to those obtained in France. We showed that the Yunnan populations differed markedly from French and JiLin populations. For all three markers, the genetic distances measured between the *Tomicus* from Yunnan and those from France were similar to distances previously observed between species. Similar distances were found between Yunnan and JiLin populations. Conversely, the distances between French and JiLin individuals were substantially lower, falling in the intraspecific range. We concluded that the individuals sampled in Yunnan belong to a new, undescribed species (*Tomicus sp. nov.*). We also showed that some individuals belong to the species *T. brevipilosus* that had never been recorded from this region before. Evolution of the genus *Tomicus* is discussed in the light of these new results.
Heredity (2004) **93,** 416–422. doi:10.1038/sj.hdy.6800518
Published online 28 July 2004

Keywords: *Tomicus*; *Pinus yunnanensis*; ITS2; 28S rDNA; mitochondrial DNA; forest pest

Introduction

The bark beetle genus *Tomicus* (Coleoptera: Curculionidae: Scolytinae) includes six species worldwide (Wood and Bright, 1992). Three are restricted to Central and Eastern China (*T. brevipilosus*, *T. pilifer* and *T. puellus*), one is found only around the Mediterranean Basin (*T. destruens*), while the remaining two species occur throughout Eurasia (*T. piniperda* and *T. minor*). The typical life cycle of any *Tomicus* species contains a phase of trunk attack and a phase of shoot attack. The trunk attack corresponds to the dispersal, mating and reproduction. Females bore a longitudinal gallery in the inner bark where they lay eggs in lateral niches. The larvae feed on the inner bark, and the complete larval development takes place on the same host. Young adults emerge after 4–8 weeks and fly to surrounding shoots where maturation feeding takes place.

T. destruens and *T. piniperda* are known to cause economic damage on pine species. However, *T. piniperda* has a larger range, and its populations in Europe and in some parts of China seem to have evolved towards different ecological strategies of host use leading to contrasting levels of forest damage. In most European countries, *T. piniperda* develops on most pine species, and may cause substantial growth losses due to the shoot feeding phase (Langström and Hellqvist, 1991). However, it very seldom kills trees (Lieutier, 1991). It is therefore regarded as a minor pest by most entomologists in Europe. On the other hand, in Southwestern China (Yunnan Province), *T. piniperda* primarily attacks *Pinus yunnanensis* and can kill healthy trees by mass attack (Ye and Zhao, 1995; Ye, 1998; Ye and Ding, 1999), causing extensive damage. The first reports of *T. piniperda* killing Yunnan pine dates back to the 1980s (Ye, 1991), and more than 200 000 ha of Yunnan pine forests have been nearly completely killed so far (Ye and Ding, 1999). This insect is therefore considered as a main forest pest in this region. Interestingly, *T. piniperda* does not cause any heavy damage in other parts of China, nor on pine species other than *P. yunnanensis*, except for some exceptional epidemics on Scots pine in North China, and on *P. armandii* in North-western China.

The Yunnan populations of *T. piniperda* present a very original behaviour, which consists of aggregation on some individual trees during the shoot maturation period. It certainly results in considerable tree weakening which could explain the heavy tree mortality due to subsequent trunk attack (Ye and Lieutier, 1997; Langström *et al*, 2002; Lieutier *et al*, 2003). This behaviour is completely unknown in Europe, where the beetle populations are quite dispersed on different trees during

Correspondence: C Kerdelhué, INRA, Laboratoire de Zoologie forestière, 33612 Cestas, France.
E-mail: Kerdelhue@pierroton.inra.fr
[4]*These authors contributed equally to this study.*
Received 30 December 2002; accepted 7 May 2004; published online 28 July 2004

shoot attack. The aggregation process has also not been described in other parts of China, such as JiLin Province. Several hypotheses can be suggested to explain the ecological differences observed between European and Yunnan populations of *T. piniperda*. One of these is that difference in host tree species is a key factor in the observed differences in insect–tree relationships. It has also been suggested that climatic characteristics of Yunnan, with no cold period between trunk and shoot attack, could partly explain the shoot aggregation phenomenon, but the possibility still remains that the Yunnan populations could differ genetically from the European ones (Ye and Lieutier, 1997). To test this possibility, we conducted a genetic study of different populations of *T. piniperda* sampled on *P. yunnanensis* in Yunnan Province in order to compare the results to the situation previously found in France (Kerdelhué *et al*, 2002). The objective was to measure the genetic divergence between Chinese and French populations of *T. piniperda* and to estimate the level of population differentiation between the two areas and understand their ecological disparities.

Material and methods

Beetle sampling

In December 1999 and January 2000, beetles were sampled on trunks or shoots of *P. yunnanensis*. Sampling was also performed in stands of *P. semaonensis* and *P. armandii*, but was unfruitful. A total of 12 sampling localities were chosen in Yunnan province, PR China, and are summarized in Table 1. The locations are shown in Figure 1. In all, 30–50 insects were collected in each locality and were immediately stored in absolute ethanol. Additionally, ca. 30 *T. piniperda* were collected in Jilin Province (JingYueTan Park, see Figure 1) on *P. sylvestris mongolia* and *P. tabulaeformis*, and six individuals of *T. minor* were sampled in six of the 12 Yunnan localities for comparison with the populations of *T. piniperda* on *Pinus yunnanensis*. The sister genus *Dendroctonus* was chosen as outgroup. Individuals of *D. frontalis* were thus sampled on *P. ponderosa* in Flagstaff, Arizona, USA in August 2002. All tubes were kept at −20°C before DNA extraction.

Beetles identification and DNA extractions

All the beetles were observed under a binocular for identification prior to DNA extraction. DNA was extracted from the head, thorax and legs of five individuals per locality as well as for the six sampled *T. minor*. The abdomen, elytras and antennae were kept apart to avoid contamination by fungi and nematodes and to permit further morphological observations. Total DNA was isolated and purified following procedures

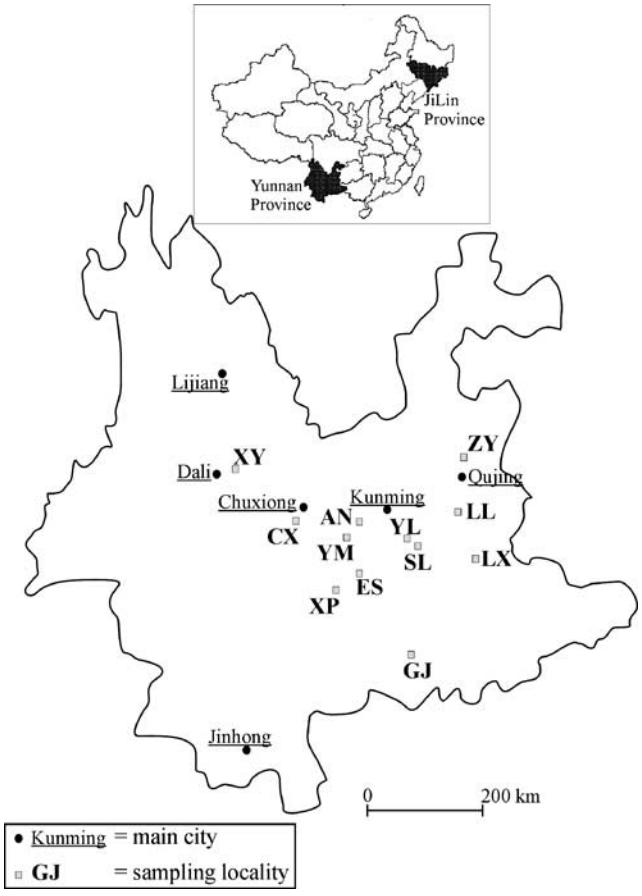

Figure 1 Map of Yunnan Province showing the main towns and the 12 sampling sites. Coding for the sampling localities are given in Table 1. The frame shows the position of Yunnan and JiLin Provinces within the PR China.

Table 1 Sampling dates and localities in Yunnan

Date	*Tree tissue sampled*	*Localities*	*No of T. piniperda (sample names)*	*No of T. brevipilosus (sample names)*	*No of T. minor (sample names)*
December 1999	Shoots	An Ning (AN)	1 (P-AN)	4 (B-AN)	0
December 1999	Trunck	E Shan (ES)	4 (P-ES)	0	1 (M-ES)
January 2000	Shoots	Ge Jiu (GJ)	5 (P-GJ)	0	1 (M-GJ)
December 1999	Shoots	Yi Men (YM)	2 (P-YM)	3 (B-YM)	1 (M-YM)
December 1999	Shoots	Xin Ping (XP)	5 (P-XP)	0	1 (M-XP)
January 2000	Shoots	Xiang Yun (XY)	5 (P-XY)	0	1 (M-XY)
December 1999	Shoots	Zhan Yi (ZY)	2 (P-ZY)	2 (B-ZY)	1 (M-ZY)
January 2000	Shoots and trunk	Yi Liang (YL)	5 (P-YL)	0	0
January 2000	Shoots and trunk	Chu Xiong (CX)	5 (P-CX)	0	0
January 2000	Shoots and trunk	Shi Lin (SL)	5 (P-SL)	0	0
January 2000	Shoots	Lu Liang (LL)	5 (P-LL)	0	0
January 2000	Trunk	Lu Xi (LX)	5 (P-LX)	0	0
2002	Shoots and trunk	JiLin Province	4 (P-JL)	0	0

from the DNeasy Tissue Kit (Qiagen) and eluted in 200 µl of AE buffer.

DNA amplification and sequencing

We used the same PCR primers as in Kerdelhué *et al* (2002) to amplify part of the cytochrome oxydase gene using either the Promega Taq package or the Sigma RedTaq (5′ CCTCATCATTATGAGCTATTGG 3′ and 5′ TCATAGGATCAATATCATTG 3′). We also amplified the nuclear ITS2 region and the D2 domain of the 28S rDNA for some individuals in order to confirm the mitochondrial results. The primers were those used by Campbell and collaborators (1993) and Lopez-Vaamonde *et al* (2001), respectively, namely ITS2F: 5′ TGTGAACTGCAGGACACATG 3′ and ITS2R: 5′ AATGCTTAAATTYAGGGGGTA 3′ for the Internal Transcribed Spacer 2, and D1F 5′ ACCCGCTGAATTTAAGCATAT 3′ and D3R 5′ TAGTTCACCATCTTTCGGGTC 3′ for the 28S. The annealing temperatures were 50°C for both COI-COII and ITS2, whereas it was 57°C for 28S rDNA. A total of 30 cycles were performed. All PCR products were then purified, either with the QIAquick PCR purification kit (Qiagen) or the GenElute PCR clean-up kit (Sigma). Purified PCR products were directly sequenced with the amplification primers. Sequencing was performed using the big-dye terminator sequencing kit and carried out either with a ABI 373 or a ABI 3100 automatic sequencer (PE Applied Biosystem).

Data analysis

The obtained sequences for each gene were aligned using Clustal W (Thompson and Higgins, 1994) as implemented in BioEdit. We first analysed all the mitochondrial sequences obtained from the Chinese beetles. To understand the high divergence measured between the Yunnan and the JiLin individuals (see Results), we then compared two individuals per group or per species with previously published sequences obtained with the same primers for French populations (Kerdelhué *et al*, 2002) of *T. piniperda* (accession numbers AF457804 and AF457825), *T. destruens* (AF457831 and AF457846) and *T. minor* (AF457865 and AF457866). We finally sequenced and analysed the ITS2 domain and D2 region of 28S rDNA for these individuals (both French and Chinese) and the outgroup *D. frontalis*. Genetic distances (JC) between the individuals as well as the numbers of transitions and transversions were calculated using *MEGA* 2.0 (Kumar *et al*, 2001). Phylogenetic trees were reconstructed with PAUP 4*b10 (Swofford, 2000) using the maximum parsimony method (MP) and maximum likelihood algorithm (ML), rooted with *D. frontalis* sequences. Separate analyses were conducted on the COI-COII and D2 data sets. The ITS2 data set was not used for the phylogenetic approach as the *Tomicus* sequences could not be aligned with the outgroup due to high sequence divergence. For MP analysis, we conducted a heuristic search with 10 random stepwise additions of sequences and tree bisection-reconnection (TBR) branch-swapping. For the ML approach, the appropriate model of evolution was chosen by Modeltest 3.06 (Posada and Crandall, 1998) and subsequently used for phylogenetic reconstructions. In all cases, a bootstrap procedure was completed with 1000 iterations for MP and 500 for ML.

Results

Beetle identification and sequence results

Morphological observation of the *Tomicus* sampled on *P. yunnanensis* showed that nine individuals out of the 68 used for molecular analyses belonged to the species *T. brevipilosus*. Identification was based on elytral setal characters following Eggers' key (Eggers, 1929), and was subsequently confirmed by Dr M Knizek (Forestry and Game Management Research Institute, Praha). The nine individuals were collected in three of the 12 sampled localities (see Table 1).

For the mitochondrial COI-COII genes, we successfully amplified and sequenced 53 individuals of *T. piniperda*, nine of *T. brevipilosus* and six of *T. minor* from Yunnan and JiLin Provinces. The length of the sequences obtained was 804 BP including 474 BP in COI, 77 in tRNALeu and 253 in COII (including alignment gaps that occur in the tRNALeu). Concerning the nuclear genes, we obtained 582 BP sequences for ITS2 and 968 BP for 28S (including alignment gaps).

All sequences have been deposited in GenBank under accession numbers AY570803–AY570903.

Genetic distances

Juke-Cantor genetic distances were calculated for all three genes (namely COI-COII, ITS2 and D2); distance ranges for within- and between group comparisons are given in Table 2. For each gene, intraspecific distances were much lower than, and did not overlap with interspecific distances. Interestingly, for all three genes, the distances between *T. piniperda* from Yunnan Province and T. *piniperda* from either France or JiLin Province were fully compatible with interspecific distances. For these reasons, we will hereafter distinguish between the Yunnan group and the JiLin group for all data analyses.

Phylogenetic reconstructions

Chinese COI-COII sequences: We first did phylogenetic reconstructions using the 68 sequences obtained on Chinese individuals (*T. piniperda*, *T. brevipilosus* and *T. minor*). We obtained 24 equally parsimonious trees of 328 steps. The 50% majority rule consensus tree is shown on Figure 2. All haplotypes from Yunnan form a monophyletic group that appears as the sister group of all other species, namely *T. minor*, *T. brevipilosus* and *T. piniperda* from JiLin, which appear in a clade.

Phylogeny of French and Chinese species and populations: We computed separate phylogenetic analyses on COI-COII and D2 sequences for a restricted data set containing only two individuals per species or group. For the COI-COII data set, the evolution model selected by Modeltest as the best fit for our data was the transversion model with gamma distribution (TVM + G: base frequencies $A = 0.336$, $C = 0.149$, $G = 0.086$, $T = 0.429$; six substitution types, rate matrix = 2.61; 24.82; 4.79; 1.71; 24.82; 1.00; gamma shape parameter = 0.185).

Table 2 Within and between group genetic distances (Juke-Cantor) for each gene studied (COI–COII, ITS2 and D2)

	T. piniperda (France)	*T. piniperda* (JiLin)	*T. piniperda* (Yunnan)	*T. brevipilosus* (Yunnan)	*T. destruens* (France)	*T. minor* (France)	*T. minor* (Yunnan)
T. piniperda (France)							
COI–COII	0–0.003						
ITS2	0						
D2	0						
T. piniperda (JiLin)							
COI–COII	0.008–0.012	0					
ITS2	0.007	0					
D2	0.001	0					
T. piniperda (Yunnan)							
COI–COII	0.118–0.121	0.108–0.121	0–0.008				
ITS2	0.122–0.126	0.127–0.131	0–0.004				
D2	0.027	0.029	0				
T. brevipilosus							
COI–COII	0.109–0.116	0.111–0.115	0.118–0.131	0–0.009			
ITS2	0.099–0.101	0.110–0.112	0.143–0.147	0			
D2	0.019	0.02	0.031	0			
T. destruens (France)							
COI–COII	0.115–0.122	0.110–0.118	0.131–0.136	0.129–0.131	0–0.006		
ITS2	0.144	0.15–0.151	0.171–0.176	0.171	0		
D2	0.039	0.04	0.039	0.043	0		
T. minor (France)							
COI–COII	0.136–0.141	0.134–0.137	0.135–0.136	0.118–0.126	0.122–0.129	0–0.01	
ITS2	0.177–0.179	0.185–0.187	0.199–0.204	0.192–0.194	0.153	0	
D2	0.057	0.058	0.048	0.059	0.043	0	
T. minor (Yunnan)							
COI–COII	0.133–0.135	0.131–0.138	0.131–0.141	0.110–0.121	0.117–0.126	0.05–0.053	0–0.009
ITS2	0.184–0.186	0.192–0.194	0.206–0.211	0.196	0.162	0.004	0
D2	0.056	0.057	0.047	0.058	0.042	0.002	0.001

In the maximum parsimony analysis, we obtained one most parsimonious tree of 390 steps. The maximum likelihood tree obtained with 500 bootstrap replicates is shown in Figure 3. Once again, the *T. piniperda* from Yunnan appear as sister group to all other haplotypes. Internal nodes are not supported, and the relative positions of the other species in the main clade are not resolved. On the other hand, the different haplotypes belonging to the same species are grouped. In particular, the *piniperda* haplotypes from France and JiLin form a strongly supported clade, as well as the *minor* haplotypes from France and Yunnan.

For the D2 domain of 28S rDNA, Modeltest selected the K80 with a gamma distribution model of evolution, with a transition to transversion ratio of 2.5 and a gamma distribution shape parameter of 0.088. The maximum parsimony approach resulted in two most parsimonious trees of 171 steps. The maximum likelihood tree obtained with 500 bootstrap replicates is shown in Figure 3. The relative positions of all species are better resolved than in the tree based on COI-COII sequences. Three strongly supported monophyletic groups appear and are grouped in a polytomy. One clade contains only the *T. piniperda* from Yunnan, a second clade groups the *T. piniperda* from France and JiLin Province with *T. brevipilosus*, and the third clade cluters *T. minor* (both from France and China) and *T. destruens*.

Discussion

Taxonomic status of *T. piniperda* from Yunnan and genus evolution

The most striking result that came out from the analysis of both mitochondrial and nuclear sequences is that the individuals from China primarily identified as *T. piniperda* are strongly structured in two clades, namely the Yunnan and the JiLin groups. The genetic distances observed within groups are fully compatible with intraspecific variation commonly observed in insects, while the distances measured between groups are similar to those previously observed between fully recognized *Tomicus* species (Gallego and Galian, 2001; Kerdelhué *et al*, 2002; Kohlmayer *et al*, 2002). Interestingly, the comparison between the two Chinese groups of *T. piniperda* and the French sequences clearly show that the distances between beetles collected in Yunnan and the French *T. piniperda* fall in typical interspecific distances (0.12 for mtDNA genes, 0.13 for ITS2 and 0.03 for D2), while the *Tomicus* from JiLin are very closely related to the French ones (genetic distance being around 0.01). It is noteworthy that quite similar results found on two of the same markers for *T. piniperda* and *T. destruens* in Spain and in France (Gallego and Galian, 2001; Kerdelhué *et al*, 2002) were used to validate the specific

status of *T. destruens*, which had previously been considered as an ecotype of *T. piniperda*. The data obtained on nuclear ITS2 and 28S rDNA being consistent with those obtained on the mtDNA genes, we can consider that the results are highly reliable. Moreover, significant ecological differences exist between the *Tomicus* from Yunnan and the European populations of *T. piniperda*. In particular, the ecology of insect–host relationships in Yunnan, with the aggregation of beetles in the shoots of one tree followed by trunk attack of this same host (Ye and Lieutier, 1997; Lieutier *et al*, 2003), is known in no other *Tomicus* species. Individuals are usually very dispersed during the maturation feeding in the shoots. Unfortunately, no data are available concerning the ecology of *Tomicus* in JiLin Province, but no substantial damage due to *Tomicus* has been reported from this Province. Based on these results, we can confidently propose the hypothesis that the individuals sampled in JiLin Province belong to *T. piniperda* while the *Tomicus* from Yunnan do belong to a new, undescribed species that develop on *P. yunnanensis*. It will be hereafter cited as *Tomicus sp. nov.* The aggregation of the beetles during the shoot maturation feeding could therefore be specific of *Tomicus sp. nov.* The new species was found only on *P. yunnanensis*, even though traps were also set in stands of *Pinus semaonensis* and *P. armandii*. *Tomicus sp. nov.* might thus be specific to *P. yunnanensis*, at least in Yunnan.

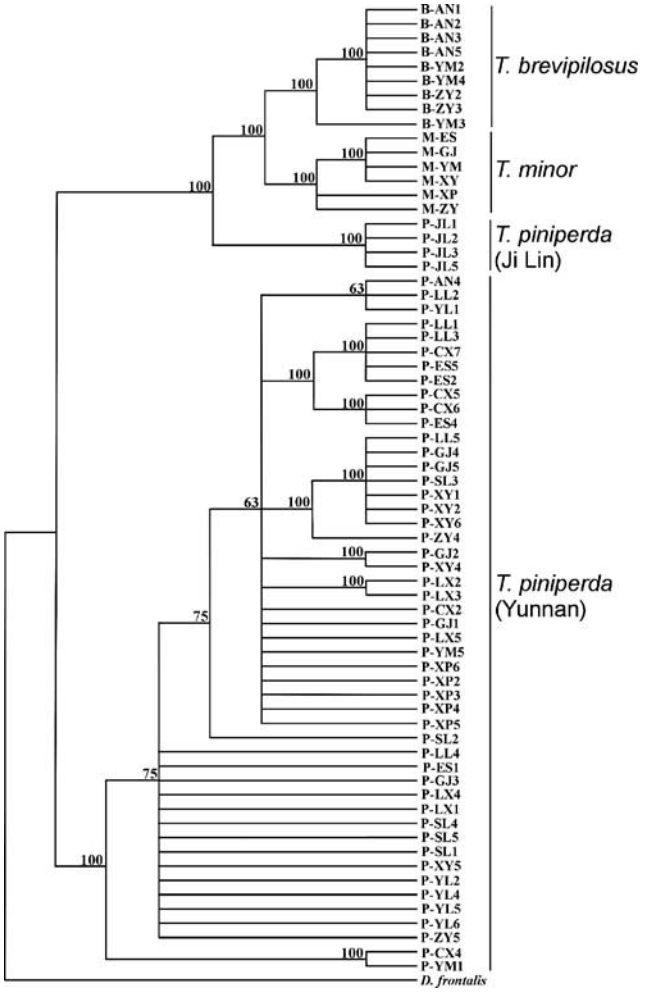

Figure 2 Phylogenetic tree reconstructed by MP analysis from the COI-COII data set of all *Tomicus* sampled in China. The tree is the 50% majority rule consensus of the 24 most parsimonious trees found by heuristic search. Coding for the Chinese beetles are given in Table 1.

Such a result has important applied consequences, if we consider the damage that occurs in Yunnan Province on *P. yunnanensis* and was previously attributed to *T. piniperda*. The biology and reproductive strategies of the new *Tomicus* species is being studied to better understand the insect–tree relationships (Lieutier *et al*, unpublished). As no *T. piniperda sensu stricto* (*ie* in its new restricted taxonomic sense) was recorded in Yunnan during the course of the present study, although our samples represented a large diversity of sites in Yunnan, it is possible that *T. piniperda* is actually absent from this Province or that its population levels are quite low. Sampling in other localities and eventually on other pine species is now necessary to determine the distribution of each species in Yunnan. A consequence is that previously published articles about *T. piniperda* in Yunnan Province (eg Ye, 1991; Ye, 1994; Ye and Zhao, 1995; Ye and Lieutier, 1997) certainly dealt with *T. sp. nov.* (that is still morphologically undistinguishable from *T. piniperda*), or with *T. brevipilosus*.

Moreover, our results show that the genus *Tomicus* actually includes seven species. Six of them are present

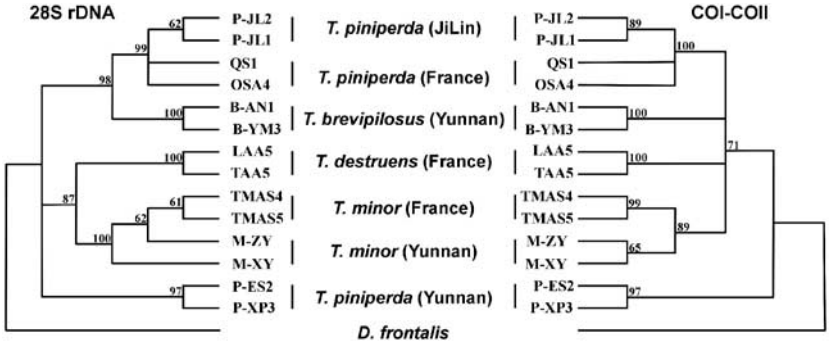

Figure 3 Phylogenetic trees obtained by ML on the D2 sequences (28S rDNA, left) and COI-COII sequences (right) on a restricted number of individuals including French species (see text for details). Numbers over each node correspond to bootstrap values obtained from 500 replicates. Coding for the Chinese beetles are given in Table 1, coding for French *Tomicus* are like in GenBank.

in Asia, among which four are probably restricted to that region of the world. Specific diversity is thus highest in Asia, which could be the centre of origin for the whole genus. On the other hand, we clearly proved that *T. destruens* is not the closest relative of *T. piniperda*, as was previously supposed earlier (Kerdelhué *et al*, 2002). The hypothesis that the two species diverged in sympatry in Europe is thus probably false. If *T. piniperda* arose in Asia and secondarily colonized Europe, the parapatry of *T. piniperda* and *T. destruens* around the Mediterranean Basin would thus rather result from a secondary contact. These evolutionary hypotheses and the biogeography of the genus *Tomicus* should be confirmed by building a more complete phylogeny of the genus, which would in particular take into account the two other Asian species (namely *T. puellus* and *T. pilifer*). A morphological revision of the whole genus is now clearly needed. Data about the geographic distribution and host range of each species will also be necessary to address evolutionary questions.

Genetic diversity and diet breadth of *Tomicus sp. nov.*

The mtDNA diversity found for *T. sp. nov.* in Yunnan province (15 haplotypes for 49 individuals) is limited, compared to that previously observed for *T. piniperda* in France (21 haplotypes for 38 individuals). However, the genetic diversity is quite similar to that found for *T. destruens* (Kerdelhué *et al*, 2002) in which nine haplotypes occurred for 34 individuals (to be compared to the 10 haplotypes found for the 49 *T. sp. nov.* on the restricted alignment of 659 BP). In a study of the sister species *Dendroctonus ponderosae vs D. jeffreyi*, Kelley *et al* (2000) found reduced genetic diversity in the specialized species as compared to the generalist, and concluded that diet breadth could play a role in the disparity of genetic diversity and structuring between species. Diet breadth and development capacity of the new *Tomicus* species found in Yunnan still needs to be confirmed, but it has been so far only found on *P. yunnanensis* despite sampling efforts done both on the shoots and the trunks of *Pinus armandii* and *P. semaonensis*. Furthermore, the local forestry bureaux confirmed that no *Tomicus* have ever been observed in Yunnan on such pine species. We can thus hypothesize that *T. sp. nov.* is specifically associated with Yunnan pine and that the restricted diet breadth could play a role in limiting the genetic diversity. This phenomenon could also result from either a historical bottleneck undergone by *T. sp. nov.*, or from smaller population sizes in that species, making it more prone to genetic drift (Whitlock and Barton, 1997). Another explanation could be that *T. sp. nov.* experiences more episodes of flushes and crashes than *T. piniperda* in France, as its populations are often epidemic on Yunnan pine (Ye and Lieutier, 1997; Ye and Ding, 1999). A similar explanation had been proposed to explain the limited genetic diversity found for *T. destruens* in France (Kerdelhué *et al*, 2002).

Acknowledgements

We are grateful to M-L Cariou (CNRS Gif-sur-Yvette) for giving us access to the automatic sequencer ABI 373, and F Maczkowiak and M Lesbats for technical assistance with this equipment. We thank M-A Auger-Rozenberg, G Roux-Morabito, J-F Silvain and D Lachaise as well as two anonymous referees for their valuable comments on the manuscript. We also thank G Roux-Morabito (Université Orléans) for advice and technical support in the laboratory. We are grateful to Lu Jun (Yunnan University), to Jianghua Sun (Chinese Academy of Sciences) and to local forestry bureaus of Yunnan province for beetle sampling. N Guerard kindly provided the *Dendroctonus* samples. We thank Dr M Knizek (Forestry and Game Management Research Institute, Praha) for his help with beetle identification, and Dr H Schönmann who kindly sent us specimens from the Natürhistorisches Museum Wien. The stays of Y Duan in France were granted by the French Government (French Embassy in Beijing).

References

Campbell BC, Steffen-Campbell JD, Werren JH (1993). Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. *Insect Mol Biol* **2**: 225–237.

Eggers H (1929). Zwei neue *Blastophagus*-Arten (Ipidae, Col.). *Entomologische Blätter* **25**: 103–104.

Gallego D, Galian J (2001). The internal transcribed spacers (ITS1 and ITS2) of the rDNA differentiate the bark beetle forest pests *Tomicus destruens* and *T. piniperda*. *Insect Mol Biol* **10**: 415–420.

Kelley ST, Farrell BD, Mitton JB (2000). Effects of specialization on genetic differentiation in sister species of bark beetles. *Heredity* **84**: 218–227.

Kerdelhué C, Roux-Morabito G, Forichon J, Chambon J-M, Robert A, Lieutier F (2002). Population genetic structure of *Tomicus piniperda* (Coleoptera: Scolytinae) on different pine species and validation of *T. destruens* (Woll.). *Mol Ecol* **11**: 483–494.

Kohlmayer B, Riegler M, Wegensteiner R, Stauffer C (2002). Morphological and genetic identification of the three pine pests of the genus *Tomicus* (Coleoptera, Scolytidae) in Europe. *Agr Forest Entomol* **4**: 151–157.

Kumar S, Tamura K, Jakobsen IB, Nei M (2001). *MEGA2: Molecular Evolutionary Genetics Analysis Software*, Arizona State University: Tempe, Arizona, USA.

Langström B, Hellqvist C (1991). Shoot damage and growth losses following three years of *Tomicus* attacks in Scots pine stands close to a timber storage site. *Silva Fenn* **25**: 133–145.

Langström B, Lisha L, Hongpin L, Peng C, Haoran L, Hellqvist C *et al*. (2002). Shoot feeding ecology of *Tomicus piniperda* and *T. minor* (Col. Scolytidae) in Southern China. *J Appl Entomol* **126**: 333–342.

Lieutier F (1991). Les coléoptères Scolytidae en France et les recherches menées par l'INRA. *Bull Soc zool Fr* **116**: 275–281.

Lieutier F, Yart A, Ye H (2003). Shoot damage by *Tomicus sp.* (Coleoptera: Scolytidae) and effect of *Pinus yunnanensis* resistance to subsequent reproductive attacks in the stem. *Agr Forest Entomol* **5**: 227–233.

Lopez-Vaamonde C, Rasplus J-Y, Weiblen GD, Cook JM (2001). Molecular phylogenies of fig wasps: partial cocladogenesis of pollinators and parasites. *Mol Phylogenet Evol* **21**: 55–71.

Posada D, Crandall KA (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

Swofford DL (2000). PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Sinauer Associates: Sunderland, Massachussetts.

Thompson JD, Higgins TJ (1994). Clustal W: improving the sensitivity of progressive multiple sequence alignment

through sequence weighting, positions specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**: 4673–4680.
Whitlock MC, Barton NH (1997). The effective population size of a subdivided population. *Genetics* **146**: 427–441.
Wood SL, Bright DE (1992). A catalog of Scolytidae and Platypodidae (Coleoptera). 2. taxonomic index. *Great Basin Nat. Mem.* **13**: 1–1533.
Ye H (1991). On the bionomy of *Tomicus piniperda* (L.) (Col., Scolytidae) in the Kunming region of China. *J Appl Entomol* **112**: 366–369.
Ye H (1994). Influence of temperature on the experimental population of the pine shoot beetle, *Tomicus piniperda* (L.) (Col., Scolytidae). *J Appl Entomol* **117**: 190–194.
Ye H (1998). Occurrence, distribution and damages of *Tomicus piniperda* in Yunnan, southwestern China. *J Yunnan Univ* **20**: 361–363.
Ye H, Ding XS (1999). Impacts of *Tomicus minor* on distribution and reproduction of *Tomicus piniperda* (Col., Scolytidae) on the trunk of the living *Pinus yunnanensis* trees. *J Appl. Entomol.* **123**: 329–333.
Ye H, Lieutier F (1997). Shoot aggregation by *Tomicus piniperda* L. (Col: Scolytidae) in Yunnan, Southwestern China. *Ann Sci For* **54**: 635–641.
Ye H, Zhao ZM (1995). Life table of *Tomicus piniperda* L. and its analysis. *J Appl Entomol* **119**: 145–148.