

Extrapolation of the melting temperatures to the inner-core boundary (ICB) at 3.3 Mbar can only yield an approximate temperature for the Earth's inner core because of the simplifications required in the absence of better knowledge. The following assumptions were made. (1) The relationship between density¹⁶ is linear because this relationship best describes previous melting experiments of alkali metals, of iron between triple points and of iron oxides¹²; and because the compression range of iron between 2 and 3.3 Mbar is only 11%. (2) There are no triple points between 1 and 3.3 Mbar in the Fe–FeO system. (3) The melting slope of pure iron above 1 Mbar is not significantly changed by the presence of other elements. (4) Eutectic melting depression plays a minor role for the outer core. Disregarding possible large uncertainties in these assumptions, this extrapolation yields a temperature of $4,850 \pm 200$ K at the ICB (Fig. 3) compared to $5,800 \pm 500$ K (ref. 1) and $7,800 \pm 500$ K (refs 2,3) based on shock measurements.

The temperature jump across the core–mantle boundary plays a key role in mantle dynamics. It can be calculated from my estimate of the ICB temperature and the adiabatic gradient across the outer core from

$$(\partial T/\partial P)s = \alpha T/C_p \rho$$

using average values for outer-core conditions of thermal expansivity $\alpha = 5 \times 10^{-6} \text{ K}^{-1}$ (ref. 5) temperature $T = 4,400 \pm 200$ K, specific heat $C_p = 3R$ (a lower limit) and density⁴. The value of $(\partial T/\partial P)_s$ does not significantly change across the liquid–solid

transition for the alkali metals at high compression¹⁷, and the volume dependence of the adiabatic gradient is the same for a large class of materials¹⁸. Therefore, the value of the adiabatic temperature drop across the outer core is calculated for solid iron, and yields 850 K. From this, the current best estimate of the temperature at the core side of the core–mantle boundary is $4,000 \pm 200$ K compared with a mantle boundary temperature of 2650 ± 100 K (ref. 12), yielding a thermal boundary layer in excess of 1,300 K. □

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Amplification and sequencing of DNA from a 120–135-million-year-old weevil

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DNA has been successfully isolated from both fossilized plant¹ and animal tissues^{2–6}. The oldest material, dated as 25–40 million years old (Tertiary), was obtained from amber-entombed bees^{4,5} and termites⁶. Tissues from both these insects yielded DNA of good quality, which could be amplified by the polymerase chain reaction (PCR) and subsequently sequenced, including the genes encoding 18S ribosomal RNA^{5,6} and 16S rRNA⁶. We report here the extraction of DNA from a 120–135-million-year-old weevil (Nemonychidae, Coleoptera) found in Lebanese amber, PCR amplification of segments of the 18S rRNA gene and the internal transcribed spacer, and the corresponding nucleotide sequences of their 315- and 226-base-pair fragments, respectively. These sequences were used for preliminary phylogenetic analysis of the nemonychid's sequence with three extant coleopterans: *Lecontellus pinicola* (Nemonychidae), *Hypera brunneipennis* (Curculionidae) and the mealworm *Tenebrio molitor* (Tenebrionidae), and two

extant dipterans: the fruitfly *Drosophila melanogaster* (Drosophilidae) and mosquito *Aedes albopictus* (Culicidae) for the purpose of ascertaining the origin of the extracted and amplified DNA. The results revealed that the PCR-amplified material is that of the extinct nemonychid weevil. This represents the oldest fossil DNA ever extracted and sequenced, extending by 80 million years the age of any previously reported DNA^{4–6}.

We report the successful isolation and nucleotide sequencing of segments of ribosomal DNA from a 120–135-Myr-old nemonychid weevil. The fossil weevil (Fig. 1) was obtained from a piece of Middle Eastern amber⁷ from beds located near the towns of Jezzine and Dar al-Baidha in Lebanon. These beds occur in primary deposits of the Neocomian epoch of the Early Cretaceous, as well as in secondary deposits in the Neocomian epoch and the Aptian stage. These deposits are dated from 120 to 135 Myr^{8,9} and represent the oldest known amber containing insect inclusions.

The weevil has been identified as belonging to the extinct subfamily Eobelinae of the family Nemonychidae in the superfamily Curculionoidea (Coleoptera), and is being described as a new genus and species¹⁰. Nemonychid weevils are the probable sister group of all other Curculionoidea (weevils) and represent an ancient group of conifer-feeding beetles^{11,12}. Of the 22 recognized genera in the Nemonychidae, all but two develop on pollen in the male cones of conifers¹³. The greatest variety of forms occurs on species of *Araucaria* and *Agathis* in the Araucariaceae and these araucarian nemonychids appear to represent the most primitive of all extant forms and most closely resemble the fossil nemonychids of the upper Jurassic Kara Tau deposits in central Asia^{13,14}. It was concluded from infrared and mass spectrometry, as well as thin-layer chromatography¹⁵, that Lebanese amber was formed from araucarian trees. Thus, it is quite possible that the fossil weevil developed in the male cones of the resin-producing tree. Only a few beetles have been repor-

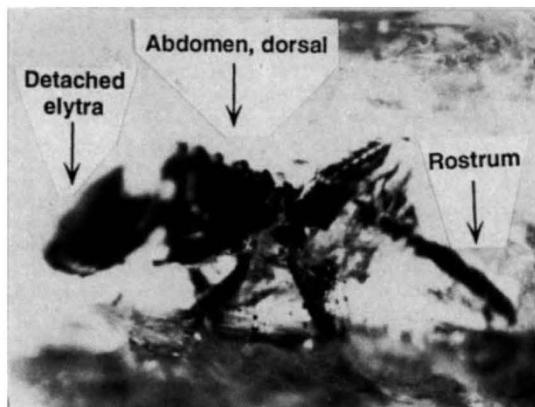


FIG. 1 Lateral view of the extinct weevil (Nemonychidae: Coleoptera) in 120–135-million-year-old Lebanese amber.

ted from Lebanese amber^{11,16} and none of them are nemonychids. In fact, nemonychids are rare in amber; none have been described and a passing mention of their occurrence in Baltic amber is the only record of this group in fossilized resin¹⁷. Today, the extant nemonychids form an amphipholar group, represented in the north and south temperate zones by disjunct groups and poorly represented in the tropics^{11–13}.

The amber piece was surface-sterilized and cracked as described⁴. During this process the amber broke open and a pellet of tissue was removed from the weevil's body cavity. DNA was extracted using Chelex-100 (BioRad), which has been used successfully to extract high-quality DNA from tissues and forensic materials for enzymatic amplification^{18,19}. DNA was also extracted from alcohol-preserved tissues of the weevil *H. brun-*

FIG. 2 Sequence alignment for a 315-base-pair sequence resulting from the 5' region of the 18S rRNA gene (*a*) and a 226-base-pair sequence from the internal transcribed spacer (ITS) of the rRNA gene (*b*) of 120–135-million-year-old weevil srRNA. Dots indicate that the base is identical to that of the outgroup *Artemia salina*. Base substitutions are indicated by the first letter of the substituting base, and gaps are indicated by a dash. Nucleotide sequences from the 18S rRNA gene used in phylogenetic inference studies are indicated by an asterisk. Sequences from the ITS region are shown with the fossil nemonychid on top to illustrate the sequence similarities between the extinct and extant weevils.

METHODS. DNA was extracted using Chelex 100 (BioRad, Richmond, CA). The pellet of tissue from the body cavity of nemonychid weevil measuring 1–2 mm in diameter and thoracic tissue from extant weevils were suspended in 500 µl of a 5% aqueous suspension and incubated for 2–4 h at 56 °C with continuous shaking. Samples were then mixed for 30 s and placed in a dry bath at 95 °C for 5 min. After centrifugation at top speed in a microcentrifuge for 5 min, the supernatant containing extracted DNA was stored at –20 °C in a sterile 0.7-µl microcentrifuge tube. For PCR amplification, extracted DNA was diluted 1:10 and 1:20 in filter-sterilized double-distilled H₂O. Aliquots (5 µl) of the diluted sample were used as the source of DNA. Symmetric PCR amplifications (1 unit of *Taq* DNA polymerase (Boehringer-Mannheim), 2 µg ml^{–1} bovine serum albumin, 0.5 µM each of forward and reverse primers, 2.0 mM MgCl₂ and 0.2 mM dNTPs in 50 µl) were reacted in a programmable thermal cycler (Ericomp, La Jolla); DNA templates were denatured for 2 min at 95 °C, followed by a primer annealing step at 48 °C for 30 s, and an extension step of 60 s at 72 °C. The following 30 cycles consisted of a 30-s denaturation step at 94 °C, a 30-s primer annealing step at 48 °C, and a 60-s primer extension step at 72 °C. The last cycle consisted of a 30-s denaturation step, a 30-s primer annealing step, and a 5-min primer extension step. The products of each amplification were evaluated by 1.2% agarose or 5% polyacrylamide gel electrophoresis. Each amplification reaction mixture (25 µl) was purified using a Magic PCR purification kit (Promega) to remove excess dNTPs, primer and buffer components. 10 µl purified PCR product was used as template in an asymmetric PCR to generate a single-stranded DNA template for sequencing. Amplification conditions were identical to those described for symmetric PCR, except that one of the primers was used at 0.5 µM and the other at 0.02 µM. The low-concentration primer was also used in the sequencing reaction. Single-stranded DNA products were sequenced using a Sequenase II DNA sequencing kit (USB, Ohio) with [α -³⁵S]dATP according to the manufacturer's instructions. Electrophoresis of sequencing products were in a 6% sequencing gel (Gel-Mix 6, Gibco). Autoradiographs of air-dried sequencing gels were made using XAR X-ray film (Kodak). Sequences were inferred from autoradiographs using a gel reader (IBI, New Haven CT).

	<i>a</i>											
<i>Artemia</i>	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****
<i>Drosophila</i>	TTATGGTTCCTC	TTAGATGCGTA	CTATATCCCA	CTTGATAAAC	TGTGTAATT	CTAGACCTAA						
<i>Aedes</i>	C...AA..TA.CA...	A.T	TA.CTAGT...				A..A					
<i>Tenebrio</i>							CCACATT...					
<i>Hypera</i>							CCACA.T...					
<i>Lecontellus</i>							CCACAA.T...					
<i>Nemonychid</i>							CCACAA.T...					
	<i>b</i>											
<i>Artemia</i>	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****
<i>Drosophila</i>	TACATGCCACA	-ATAGCCCA	ACTTC-ACG	GAAGGGGTGC	TTTTATTAGA	TCAAGACCAA						
<i>Aedes</i>AT.T.A.A.ATG...C.T...T...G.C.T...	G	C.T...	A						
<i>Tenebrio</i>A...AT...AGG...C...G...CCT...AA...T.C...A...							
<i>Hypera</i>A.C.G...T...G...CGGAG...A.C...A.C...A...							
<i>Lecontellus</i>A.C.G...T...G...CGGAG...A...A...A...							
<i>Nemonychid</i>A.C.G...T...G...CGGAG...A...A...A...							
	<i>a</i>											
<i>Artemia</i>	TCGG-GGC--	TTCGGCTCGT	CT-----	CTTGGTCACT	CTGAAATACT	ATA-GCCGAT						
<i>Drosophila</i>	G...ATC..AA G-----AGTTATATTG	G...A-----AGA T-----A							
<i>Aedes</i>TCTT.CG-----GCTGGA	G...A-----AG...T...T.C...T...						
<i>Tenebrio</i>T...GG...CTC...ATCGTACAA										
<i>Hypera</i>T...GG...G.TA...ATCGTACAA										
<i>Lecontellus</i>T...GGG.G.TTA...ATCGTACAA										
<i>Nemonychid</i>T...GG.G.TTA...ATCGTACAA										
	<i>b</i>											
<i>Artemia</i>	CGCACCGCTCT	CGCACCGGGG	ACCGTGTCTT	CAAATGTCG	CCCTATCAC	TTTCGATGTCG						
<i>Drosophila</i>T.C...A.G...T.A.C...T.C.A...TAA...GGAA	TC...G.TTC.	AT...CC.G.GA					
<i>Aedes</i>T.A...GAG...T.A.C...T.C.A...TAA...GGAA	TC...G.TTC.	AT...CC.G.GA					
<i>Tenebrio</i>T.C...G...T.C.A.C...T.C.A...TAA...GGAA	TC...G.TTC.	AT...CC.G.GA					
<i>Hypera</i>T.C...G...T.C.A.C...T.T.A...TAA...GGAA	TC...G.TTC.	AT...CC.G.GA					
<i>Lecontellus</i>T.C...G...T.C.A.C...T.T.A...TAA...GGAA	TC...G.TTC.	AT...CC.G.GA					
<i>Nemonychid</i>T.C...G...T.C.A.C...T.T.A...TAA...GGAA	TC...G.TTC.	AG...CC.G.GA					
	<i>a</i>											
<i>Artemia</i>	AGGCTATCGG	ACGGGTTAACG	GGGAATCCGG	GTTCGATTC	GGAGACGGAG	CCTGAGAAC						
<i>Drosophila</i>TA.C...AG...T.A.C...T.C.A...TAA...GGAA	TC...G.TTC.	AT...CC.G.GA					
<i>Aedes</i>TA...GAG...TA.C...T.C.A...TAA...GGAA	TC...G.TTC.	AT...CC.G.GA					
<i>Tenebrio</i>T.C...G...T.C.A.C...T.C.A...TAA...GGAA	TC...G.TTC.	AT...CC.G.GA					
<i>Hypera</i>T.C...G...T.C.A.C...T.T.A...TAA...GGAA	TC...G.TTC.	AT...CC.G.GA					
<i>Lecontellus</i>T.C...G...T.C.A.C...T.T.A...TAA...GGAA	TC...G.TTC.	AT...CC.G.GA					
<i>Nemonychid</i>T.C...G...T.C.A.C...T.T.A...TAA...GGAA	TC...G.TTC.	AG...CC.G.GA					
	<i>b</i>											
<i>Nemonychid</i>	CGCTCTCGTA	GTGAACCTGC	GGAGGGATCA	TTTATTGCGT	TGCATCAACC	AGGAATCTT						
<i>Lecontellus</i>ATC...T...T...A.T.C...T...AA...T...T.A...T...AA...T...GG...					
<i>Hypera</i>G.T...C...TCCTT.AT...AT.CA.GACCC...G...AA...G...A...TC...				
<i>Drosophila</i>TTC.GTG...GAACCTG.G.AGATC-ATTA...GTTATAT.C...CTTACC...TTAA.A					
<i>Aedes</i>G...T...T...T...T...T...T...T...				
<i>Nemonychid</i>	CGGATTGTCT	GCACCAACTG	ACATTTGTCGA	GGGGTCAACA	TTGGAAATGGT	GTATTTTAA						
<i>Lecontellus</i>				
<i>Hypera</i>				
<i>Drosophila</i>AAT...GTGT...AT...-ACA...G...A...T...GG...G...G...G...				
<i>Aedes</i>GA...-G...T...T.A...G...T...T.A...G...T...G...G...				
<i>Nemonychid</i>	TTTATTCTCA	TCTTACATG	TGAGACACAA	TTTGAGATTA	ATCTTCACAA	CTTTCACAA						
<i>Lecontellus</i>G...C...T...T...T...T...T...T...				
<i>Hypera</i>T.G...G.C...T...A.T...C...G...G...G...				
<i>Drosophila</i>CT...A...AAA...A ATT...TIG...ACCT...T.C...CA-AA.C...CC.AGG...G					
<i>Aedes</i>CT...A...AAA...A ATT...TIG...ACCT...T.C...AA.A...G...AT...AT...C.A.G.G...				
<i>Nemonychid</i>	CGGATCTCTT	GGTCTCTCCA	TGCGATGAGA	ACCCACCAA	CTGGGT							
<i>Lecontellus</i>C...C...T...T...T...T...T...T...				
<i>Hypera</i>T...GA...AG...T...T...G...G...G...G...G...				
<i>Drosophila</i>G...A...C...G...C...G...T...C...T...A...C...T...A...C...				
<i>Aedes</i>T...A...C...A...T...GG...T...A...T...G...T...G...				

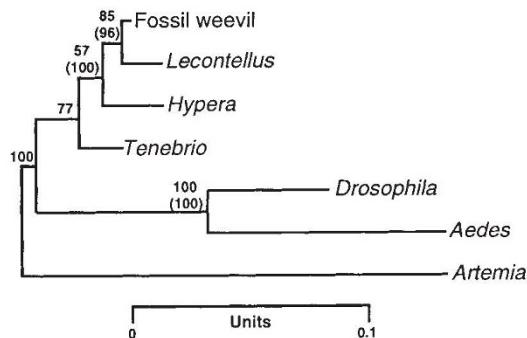


FIG. 3 Phylogenetic tree, obtained by Neighbor Joining, of extinct and extant taxa based on sequences of the 18S rRNA gene showing bootstrap confidence values. Trees were obtained by Neighbor Joining (NJBOOT2 and Treeview), Maximum Likelihood (DNAML), and Maximum Parsimony (PAUP 3.0s). All three trees had identical topology. The numbers at the nodes of branches represent bootstrap confidence values obtained from 2,000 replications²⁶. Numbers in parentheses are the bootstrap confidence values obtained from Neighbor Joining analysis when ITS sequences in Fig. 2b were appended to the 18S rRNA sequences (Fig. 2a). Corrections for transitions and transversions were made using the Kimura 2 parameter distance method²⁷. Maximum Likelihood tree was constructed with the DNAML program of PHYLIP 3.4 (ref. 23) using the brine shrimp *Artemia salina* as the outgroup, with randomized data input (*J* option), and global rearrangement of data (*G* option). A total of 6 independent runs were evaluated. All resulting trees were identical. Length of Maximum Parsimony tree by Branch and Bound method was 125. Consistency index = 0.968, or excluding uninformative sites, 0.915.

neipennis and dried museum specimens of the extant nemonychid *L. pinicola* in order to provide amplifiable DNA for comparative studies. No DNA was extracted from extant species until after the DNA from the fossil nemochid had been extracted, amplified and sequenced.

Extracted DNA was used as a template using a panel of primers whose sequences were derived from conserved regions of eukaryotic 18S rRNA corresponding to the 5' end of the 18S rRNA gene (NS1 + NS2) (ref. 20) and the internal transcribed spacer (ITS1 + ITS2)²¹. Amplified DNA of the expected size (see ref. 21) was obtained from the fossil DNA with each primer pair used. As bacterial DNA either does not amplify with eukaryotic primers or gives fragments of a different size, our results support the hypothesis that the amplified DNA was of eukaryotic origin.

Single-stranded templates from both these amplified ribosomal gene products were generated by asymmetric PCR²² and sequenced using the dideoxy termination method. For comparative analysis, sequences consisting of 315 base pairs from the NS1 + NS2 amplified product and of 226 base pairs from the ITS1 + ITS2 were chosen to evaluate the nemonychid origin of the extracted and amplified DNA.

These sequences were used to search the GenBank database using the IBI/Pustell DNA analysis program. The highest similarity scores were obtained with all insect sequences present in the database: the coleopteran *Tenebrio molitor* (accession number, X07801) and two dipterans: *Drosophila melanogaster* (accession numbers, M21017 and M29800) and *Aedes albopictus* (accession number X57172). An even higher score was obtained when the nemonychid weevil sequence was compared with those obtained from the extant weevils *L. pinicola* and *H. brunneipennis*. Significantly lower scores were seen with human and bacterial small RNA, demonstrating that the amplified DNA was of insect origin, and probably from curculionid insect origin. Alignment of the nemonychid weevil small rRNA sequences with the five insect sequences already mentioned is shown in Fig. 2.

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