What does *Cryptocercus kyebangensis*, n.sp. (Dictyoptera: Blattaria: Polyphagidae) from Korea reveal about *Cryptocercus* evolution? A study in morphology, molecular phylogeny, and chemistry of tergal glands

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ABSTRACT—The description of a new species of the woodroach *Cryptocercus kyebangensis* Grandcolas from South Korea offers the opportunity to bring comparative information within the genus. This species, though morphologically very similar to other East Asian and North American species, presents conspicuous differentiation of both ribosomal genes (sequenced fragments of 12S and 16S) and chemical blends from tergal glands (proportions of linalyl acetate and the alcohol 4, 6, 8-trimethyl-7, 9-undecadien-5-ol, compounds previously identified in females originating from North America). A phylogenetic reconstruction involving *Blatta orientalis* as an outgroup, *Therea petiveriana* as a polyphagid relative, *C. kyebangensis* and 17 North American *Cryptocercus* populations showed that *C. kyebangensis* stands as a sister-group of North American *Cryptocercus*, thus suggesting that one beringian vicariance has taken place in the early differentiation of the genus. Using sequence divergences within the framework of the molecular clock leads to comparable datation estimates for both ribosomal genes, ranging between Miocene and Pliocene for the whole history of the genus *Cryptocercus*, therefore congruent with previous phylogenetic biogeographical estimates. This new set of data for this East Asian species supports heterobathmy in the genus *Cryptocercus* and argues against the misleading "primitive taxon" concept.

SPECIES NOVA: *Cryptocercus kyebangensis* Grandcolas.

INTRODUCTION

The woodroach *Cryptocercus* Scudder, 1862 has been considered for a long time as a key taxon for understanding relationships and evolution in Dictyoptera and Isoptera because, similar to the so-called "lower" termites, it is xylophagous, subsocial and harbors intestinal protista (Cleveland et al., 1934; Grassé and Noirot, 1959; Nalepa, 1984; Grandcolas and Deleporte, 1992, 1996; Grandcolas, 1997, 1999a).

First described from North America (Scudder, 1862), the genus *Cryptocercus* was also known early from East Asia (Bey-Bienko, 1935, 1938, 1950). However, most reports dealing with *Cryptocercus* and related information potentially usable for comparative and evolutionary studies focused on the North American populations (*e.g.,* Cleveland et al., 1934; Seelinger and Seelinger, 1983; Hamilton et al., 1985; Nalepa, 1984, 1988; Nalepa and Mullins, 1992; Appel, 1989; Gäde et al., 1997), which have been recently shown to comprise several species (Kambhampati et al., 1996; Nalepa et al., 1997; Burnside et al., 1999a). East Asian populations have only been studied for their habitat and in-

testinal protista (Bey-Bienko, 1950; Mamaev, 1973; Bobyleva, 1975; Grandcolas, 2000). This biased state of knowledge is obviously detrimental to the generalization of evolutionary hypotheses which could change according to original characters shown by East Asian taxa. The same way, biogeographical hypotheses need to take into account East Asian *Cryptocercus* species to be significantly supported (Grandcolas, 1999b, 1999c). In this context, a point which has never been questioned until now is the monophyly of North American *Cryptocercus* relative to East Asian ones. Another interesting information would be an estimated date of splitting between *Cryptocercus* of both regions obtained with a molecular clock; this point is more debatable, however, depending upon the confidence one puts in evolutionary clocks in general.

According to Bey-Bienko (1950), the genus *Cryptocercus* is known from a wide range of temperate forests in China, Russia and Korea. To increase knowledge on East Asian species, behavioral and ecological studies have been initiated on Korean populations (Y.C. Park and J.C. Choe, in prep.). These populations were known until now by a few museum specimens and were

attributed to the species described earlier from China, *C. primarius* Bey-Bienko, 1938, in the most recent literature (Asahina, 1991). Extensive field sampling first by Yung Chul Park, and later by Yung Chul Park and Philippe Grandcolas, have shown that some *Cryptocercus* populations exist in most of the forested regions from the north (Mt. Chŏmbong, 1424 m, 23 km southwest of Sokch'o) to the south (Tŏgyusan National Park, 54.5 km east from Chonju) of South Korea.

The present study provides comparative information on East Asian *Cryptocercus* using a new species described from South Korea. This species is described from the point of view of its morphology. Its molecular phylogenetic position as well as its sequence divergence are analyzed to provide a first test of the monophyly of North American populations and to estimate the time of splitting between basal sister-groups within the genus. Its tergal glands are also studied to assess if their products significantly vary from Asian and North American species.

MATERIAL AND METHODS

Field Sampling and Morphological Studies

Specimens were sampled in the field in October 1998 by Y.C. Park and P. Grandcolas, by opening dead trunks and branches in the understory of temperate Korean forests. Most specimens were then dried and kept in collections on pins, but some were preserved in absolute ethanol to permit subsequent sequencing or anatomical observations. Male and female genitalia (including spermathecae) were dissected out with micro-scissors and forceps and observed after clearing with cold KOH. They are conserved in glycerin in tubes pinned beneath the specimens. Genitalia nomenclature is taken from Grandcolas (1996), modified from McKittrick (1964). Measurements are given according to specimens listed in the description.

Abbreviations are: ANSP (Academy of Natural Sciences of Philadelphia), MNHN (Muséum national d'Histoire naturelle, Paris), and NMNHK (National Museum of Natural History, Korea).

DNA Sequencing

Ribosomal genes of the new Korean species of *Cryptocercus* described below, and *Therea petiveriana* (Polyphagidae), were studied. Partial sequences of 12S and 16S rRNA, equivalent to those reported by Kambhampati et al. (1996) for North American *Cryptocercus*, were sequenced. Genomic DNA was extracted from the musculature of 3 legs of a dried specimen. Legs were homogenized in 100μl of buffer (10mM Tris-HCl pH 9, 0.1M EDTA, 1% SDS and proteinase k 0.2mg/ml) with a sterile plastic pestle. The homogenate was incubated for 4 h at 50 °C, and DNA was precipitated with the phenol-chloroform method, then genomic DNA was resuspended in sterile water.

Polymerase chain reaction was carried out in a thermal cycler (MJ Research, Inc.) using a Taq Expand polymerase (Roche). PCR conditions were: 94° C for 3 min followed by 35 cycles of 94° C for 30 sec, 55° C for 1 min, 72° C for 1.5 min, and finally an extension period of 72° C for 10 min. The amplified product was electrophoresed on a 1.2% agarose gel. PCR-amplified DNA fragments were cloned into a p-GEM-Teasy vector (Promega). Clones were sequenced on both strands in an automated fluorescence sequencing system ABI (Perkin Elmer).

The primers used to amplify a fragment of 12S rRNA (~430 bp) and 16S rRNA (~415 bp) were those described by Kambhampati (1995) and Kambhampati et al. (1996). The four sequences of two species have been deposited in the EMBL Nucleotide Sequence Database under accession numbers AJ294933 and AJ294932 (12 S and 16S of *Therea petiveriana*) and AF310221 and AF310220 (12 S and 16S of the new Korean *Cryptocercus*).

Sequence Alignment and Phylogenetic Analysis

The sequences here obtained were analyzed together with those retrieved from GenBank, including 17 North American populations (Kambhampati et al., 1996) and *Blatta orientalis* (Kambhampati, 1995). *Therea petiveriana* (Polyphagidae), previously inferred to be closely related to the genus *Cryptocercus* (Grandcolas, 1993, 1994a; Gäde et al., 1997), was included in the sample to test the monophyly of the genus *Cryptocercus*. *Blatta orientalis* (Blattidae) was used as an outgroup. The alignment and the cladistic analysis of 12S and 16S sequences together were carried out using the program POY (Gladstein and Wheeler, 1997) which implements the "direct optimization" method of Wheeler (1996). This method analyzes the number of sequence transformations implied by a phylogenetic tree and does not require multiple sequence alignment. The program, computationally demanding, was run on a PC-compatible microcomputer with 128 MB of RAM, and a 500 MHz Pentium® processor. We used the command list "-seed -1 -random 15 -multibuild 15 -buildslop 5 -slop 5 -checkslop 10 -ratchettbr 50 -ratchetpercent 70 -ratchetseverity 2 -ratchettrees 2." A brief sensitivity analysis (*sensu* Wheeler, 1995) was carried out using five contrasted parameter sets (gap: transversion: transition): 1 2 2, 1 1 1, 2 1 1, 3 1 1, 10 1 1 to test how robust the results are against parameter changes. The set 2 1 1 is considered the most standard for a wide range of organisms (*e.g.,* Giribet and Ribera, 2000). The most parsimonious tree(s) and their length were thus obtained directly with the program POY.

Datations are tentatively made according to molecular clock hypotheses, taking into account half-values

of sequence divergence in both sequenced genes (without model or distance corrections) and several substitution rates given in the literature for diverse mitochondrial genes. When sister-groups comprise more than one taxon, the mean of sequence divergence from among a group is computed if the variance within values is lower than 5%.

Chemical Analysis of Glands

Two pools of four adult females originating from Korea (Kyebang Mountain, 1577 m, 38 km westsouthwest of Kangnŭng, and Tŏgyusan National Park, 54.5 km east from Chonju) were analyzed. Age and social status of these females were unknown. The tergal gland—underlying tergite 7 (Farine et al., 1989)—was dissected from each female with microscissors and forceps and the gland was removed from the adjacent tissues. The glands were pooled in a vial of 5 ml distilled pentane and kept at −20°C until used. Just before analyses, the glands were removed from the solvent and the extract concentrated to 50 μl using a gentle nitrogen stream.

GC analyses of extracts were performed using a Hewlett Packard® 437 A fitted with a flame-ionization detector. A CP Sil 5CB (30 m ×0.25 mm i.d., 0.22 μm film thickness, Chrompack) fused silica capillary column was used for analyses. Two microliters of sample were injected into a split-splitless injection system, operating with a split flow of 25 ml/min and a septum purge of 3 ml/min. The split port was closed during injection and then opened 30 sec after injection. The column was held isothermally at 40° C for 2 min, then programmed to increase at a rate of 20° C/min for one min, 2° C/min to 154° C and then 10° C/min to 250° C. Hydrogen was used as carrier gas (50 cm/sec velocity at room temperature). The injector and detector temperatures were 250° and 270° C, respectively. Collection of the data was done automatically by PC software (Maestro, Chrompack).

GC-MS was carried out using a Nermag R 10-10 C quadrupole mass spectrometer coupled with a Girdel 31 gas chromatograph. The GC conditions were the same as those just described. The column was connected directly to the ion source of the spectrometer through a heated transfer line maintained at 260° C. Electron impact (EI) mass spectra were obtained at 70 eV with a 0.8 sec cycle, the instrument scanning from 25 to 300 amu with a source temperature of 150° C. Chemical ionization (CI) spectra, using ammonia with a source pressure of 0.3 Torr, were obtained at 90 eV with a source temperature of 90°C, the instrument scanning from 60 to 300 amu in 0.7 sec.

Compounds were identified by comparing their spectra to those published elsewhere (Le Quéré et al., 1991). Correct identification was checked by co-analyzing the biological peaks and their corresponding synthetic compounds (linalyl acetate, Interchim, France; 4, 6, 8-trimethyl-7, 9-undecadien-5-ol, gift of Dr K. Mori, Japan) under the same conditions on CP Sil 5CB column.

SYSTEMATICS

Cryptocercus kyebangensis Grandcolas, **n. sp.** (Figs. 1–3, 6, 8–10, 15, 16, 19)

HOLOTYPE.—SOUTH KOREA: Kyebang Mountain (= Kyebangsan, = Gyebangsan), 1577 m, 38 km westsouthwest of Kangnŭng; temperate deciduous forest, 3.X.1998 (Yung Chul Park and Philippe Grandcolas) (MNHN); male.

ALLOTYPE.—Type locality; female.

PARATYPES.—Type locality: 6 males and 1 female (pinned), 4 males and 6 females (in alcohol) (MNHN); 2 males and 2 females (in alcohol) (ANSP); 3 males and 3 females (pinned) (NMNHK). Sokyebang Mountain (= Sokyebangsan), 1456 m, 32 km west of Kangnŭng, temperate deciduous forest, 3.X.1998 (Yung Chul Park and Philippe Grandcolas) (MNHN), 1 male and 2 females.

Other Specimens.—SOUTH KOREA: Kyebang Mountain (=Kyebangsan), 1577 m, 38 km west-southwest of Kangnŭng, temperate deciduous forest, 3.X.1998 (Yung Chul Park and Philippe Grandcolas) (MNHN); two larvae (pinned) and 5 larvae (in alcohol). Sokyebang Mountain (= Sokyebangsan), 1456 m, 32 km west of Kangnŭng, temperate deciduous forest, 3.X.1998 (Yung Chul Park and Philippe Grandcolas) (MNHN), 3 larvae.

Etymology.—This species is named after the type locality. According to newer Romanized spellings of Korean names, the type locality should be now spelled Gyebang Mountain, not Kyebang Mountain. We, however, prefer to name this new species according to the previous spelling, which is still prevalent in geographical data bases and maps.

Description.—Small-sized species, not slender but wide and not cylindrical but quite flattened dorso-ventrally by comparison with other species of the genus; coloration black or very dark brown, even on the ventral side.

Pronotum (Fig. 1) smooth, with some low ridges or small projections but without horns or sharp projections, and not very developed laterally; its cephalic margin shaped as a carina only slightly not widened in the middle and slightly concave.

Fore femora (Fig. 2) with four, rarely three or five, apical spines on the ventro-anterior margin; all femora with one (sometimes two) spines on the ventro-posterior margin; all spines moderately long and robust.

Abdomen with tergites smooth, with few and very weak punctuations and without any other protuberances; large tergal glands similar to those described in
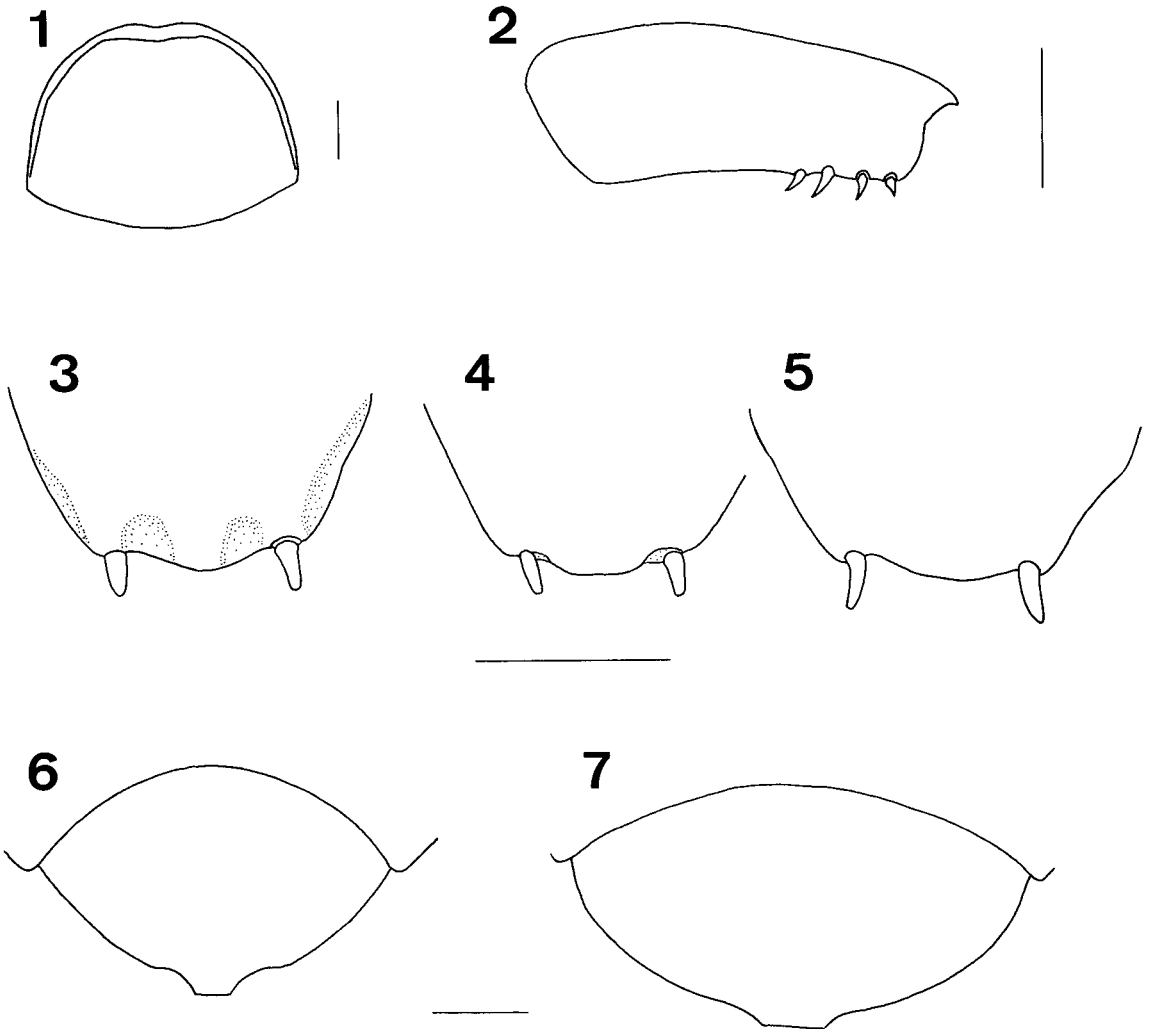
Figs. 1–7. *Cryptocercus* spp. **1–3, 6,** *C. kyebangensis* Grandcolas, n.sp.: **1,** Male pronotum; **2,** left fore femur (cephalic view); **3,** Male subgenital plate with pits indicated by dotted areas; **6,** Female subgenital plate. **4,** *C. relictus*, male, HOLOTYPE, subgenital plate. **5, 7,** *C. primarius*, female, HOLOTYPE, subgenital plate. Scale bars = 2 mm.

C. punctulatus (Farine et al., 1989); covered eighth sternite without projections on the caudal margins. Sternites also smooth.

Male subgenital plate (Fig. 3) with caudal margin regularly rounded and very slightly protruding in the middle, between the two well-differentiated styli which are apically rounded; with weak concavities on each lateral margin and inner to each stylus. Male supra-anal plate subtriangular with a clearly rounded caudal margin.

Female subgenital plate (Fig. 6) with a narrowly truncate apex and convex suabpically.

Male Genitalia (Figs. 8–10).—Apical and dorsal part of sclerite L2v deeply sclerotized (Fig. 8); ventral and elongated part of the same sclerite with the basal part irregularly quadrate, and the apical part medium-sized and ending with a widened sclerotization (Figs. 9–10);

the apical forked part of the sclerite R3d with two branches the ventral one is wide and rounded and the dorsal one is straight and narrow, separated by a small rounded unsclerotized space (Fig. 8). Ventral phallomere very weakly sclerotized.

Female Genitalia.—Inner side of basivalvulae (Fig. 16) straight or evenly rounded but evenly sclerotized; laterosternite IX with the ventral arm digitiform, and the dorsal margin not sinuous (Fig. 15). Spermatheca (Fig. 19) with short ducts, the basal one clearly widened before the fork leading to a big and oval terminal ampulla and a small and elongated basal ampulla; the length of the duct leading to the terminal ampulla is highly variable.

Ootheca.—Similar to the ootheca described from *C. punctulatus.*

Measurements (mm, females in parentheses).—
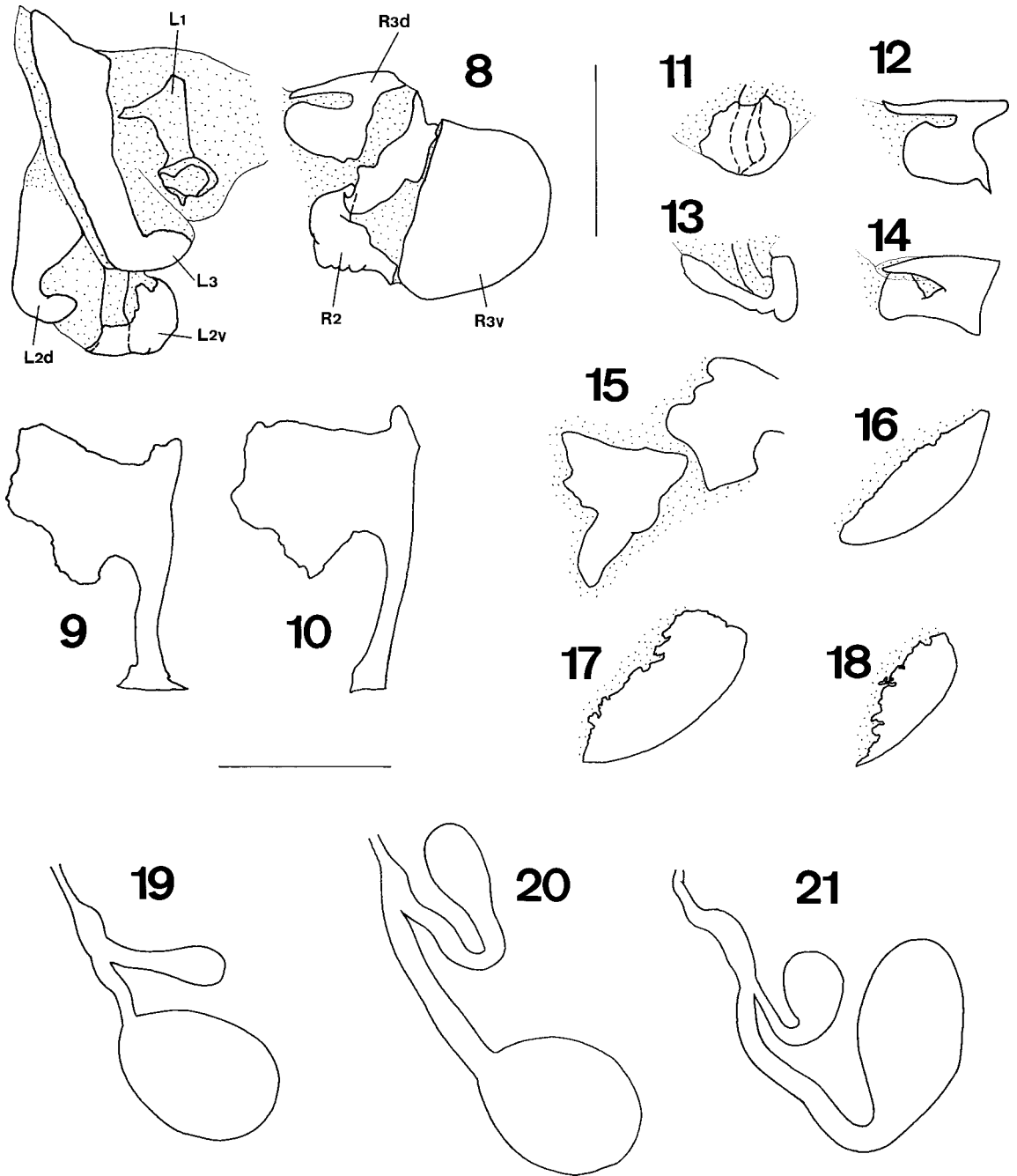
Figs. 8–21. *Cryptocercus* spp. **8–10,** *C. kyebangensis* Grandcolas, n.sp.: **8,** Male genitalia, dorsal view of the left phallomere and caudal view of the right phallomere; **9–10,** ventral view of the sclerite L2v of male genitalia, two different specimens. **11, 12,** *C. relictus*, male HOLOTYPE: **11,** dorsal view of the apex of sclerite L2v of male genitalia; **12,** caudal view of the sclerite R3d of male genitalia. **13, 14,** *C. primarius*, male: **13,** dorsal view of the apex of sclerite L2v; **14,** caudal view of the sclerite R3d of male genitalia. **15, 16,** *C. kyebangensis*, n.sp., female: **15,** laterosternite IX of female genitalia; **16,** right basivalvula of female genitalia. **17,** *C. relictus*, ALLOTYPE: right basivalvula of female genitalia. **18,** *C. primarius*, HOLOTYPE, right basivalvula of female genitalia. **19,** *C. kyebangensis*, n.sp., spermatheca. **20,** *C. relictus*, ALLOTYPE, spermatheca. **21,** *C. primarius*, HOLOTYPE, spermatheca. Scale bars = 1 mm (except 0.5 mm in Fig. 15).

Length, 17–19.5 (17.5–19); pronotum length, 3.9–4.8 (4–4.4); pronotum width, 6.2–7.4 (6.5–6.6).

Comments.—This population was collected in a small valley the bottom and the slopes of which were covered of deciduous forest. Six other distinct populations of *Cryptocercus* have been sampled throughout the whole territory of South Korea but were not included in this account because they show morphological variation which suggests that they are not conspecific with *C. kyebangensis*. We prefer to base the present description on material the homogeneity of which is unquestionable and which represents the populations used for behavioral studies. Further caryological and molecular studies will be necessary to assess how many species are present in South Korea.

Behavioral Ecology.—This species was found in rotten trunks and branches, mostly on the ground, and it harbors intestinal protista as *C. punctulatus*, *C. clevelandi* or *C. relictus* (Cleveland et al., 1934; Bobyleva, 1975). Similarly, families or old solitary individuals shelter in galleries and chambers. The abundance of this species is however much lower than for *C. punctulatus*; the same sampling effort in the understory resulted in much smaller samples, about one fifth as many (P. Grandcolas, personal observation) but a statistical sampling analysis is needed to assess whether it is related only to specific population parameters or both to these parameters and to the differential abundance or distribution of the dead wood. Such low density is in accord with the general paucity of subcortical arthropods in South Korean forests reported by Choe (1997).

Comparison with Other Species.—This species is quite distinct from others described from North America and the Old World, although examination of genitalia is needed for identification. We provide criteria for the identification of *C. kyebangensis*, which have proved to be very specific of this species by comparison with samples from different locations in East Asia and North America belonging to different species.

C. kyebangensis differs from North American species mainly by its small size (generally less than 20 mm), by obviously lacking punctuations on the last tergites and projections on the male covered eighth sternite, by having a male subgenital plate with a caudal margin regularly rounded between the styli and having a very slightly medially concave pronotal cephalic margin.

C. kyebangensis differs from *C. relictus* (Russia) by having spines on the apex of the ventro-posterior margins of middle and hind femora, by having a caudal margin of the male subgenital plate regularly rounded between the styli (Fig. 3 versus Fig. 4) and by genitalic characters: male sclerite R3d with the outer part not very widened and not shorter than the inner part (compare Fig. 8 with Fig. 12), male sclerite L2v stronger and deeper (compare Fig. 8 with Fig. 11) female basivalvulae with the inner margin more evenly sclerotized (compare Fig. 16 with Fig. 17), and the spermatheca

Table 1. Nucleotide composition in the partial sequences of the 12S and 16S ribosomal genes studied in *C. kyebangensis* compared to the means for North American populations of *Cryptocercus* (Kambhampati et al., 1996), indicated within parentheses. Sequence length is given without gaps (prior to alignment).

Base	12S	16S
Length	394 (440)	405 (406–414)
Adénine	46.4 (45.0)	43.2 (43.0)
Cytosine	17.0 (16.2)	17.0 (17.3)
Guanine	9.9 (9.9)	10.6 (10.1)
Thymine	26.7 (28.9)	29.2 (29.6)

with the basal ampulla elongated, not oval-spherical (compare Fig. 19 with Fig. 21).

C. kyebangensis differs from *C. primarius* (China) by its small size, by lacking any rugosities or punctuations on the tergites, by the female subgenital plate with well-curved margins lateral to the apex (compare Fig. 6 with Fig. 7), by genitalic characters: male sclerite L2v with an apical and dorsal part deeply sclerotized instead of apically and widely (compare Fig. 8 with Fig. 13), male sclerite R3d with the outer part rounded and moderately elongated and not robust and fork-like (compare Fig. 8 with Fig. 14), spermatheca with the basal ampulla elongated but not oval (compare Fig. 19 with Fig. 20), and the female basivalvulae slender with the inner margin evenly sclerotized (compare Fig. 16 with Fig. 18). *C. kyebangensis* also differs from *C. matilei* (China) by the same characters (except for characters in male genitalia inapplicable because the male sex is undescribed in *C. matilei*).

When *C. kyebangensis* is compared to undescribed populations from other regions of South Korea, the male and female subgenital plate, the male sclerites L2v and R3d, and the spermatheca appear diagnostic for the populations ranging in Mountains Kyebang and Sokyebang.

RIBOSOMAL SEQUENCES AND PHYLOGENETIC ANALYSES

The sequences obtained are quite similar as a whole to those of North American populations (Table 1, Appendix 1 and 2). Their analysis with diverse parameter sets provide us with basically the same tree topology. This basic and robust topology is also found when 12S and 16S sequences are analyzed separately. Tree lengths vary from 602 steps (1 1 1) to 1296 steps (10 1 1). The consensus of the four most parsimonious trees obtained with the most standard parameter set (2 1 1) is figured (Fig. 22). In all trees, *Therea petiveriana* stands as the sister-group of the genus *Cryptocercus*, and *C. kyebangensis* as the sister-group of North American sam-
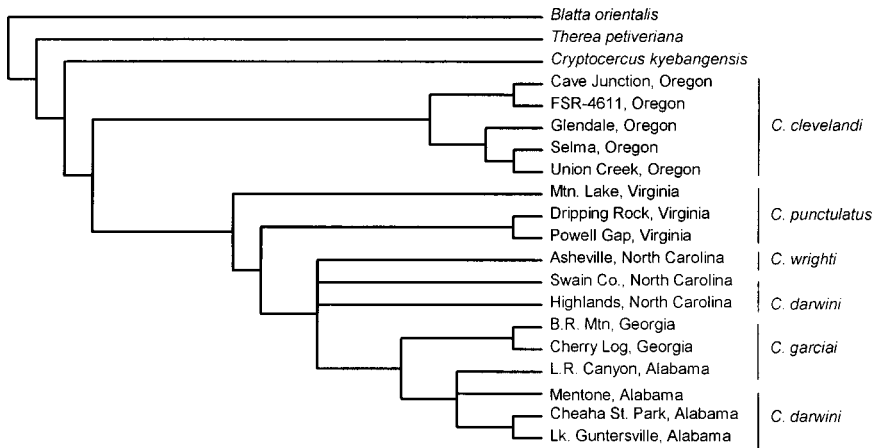
Fig. 22. Consensus of the four most parsimonious cladograms obtained from the direct optimization procedure carried out by the program POY using the sequences of 12S and 16S ribosomal genes (parameter set 2:1:1). *Blatta orientalis* was used as an outgroup. Tree length is 708 steps.

ples, demonstrating that the genus *Cryptocercus* and North American samples respectively, are monophyletic with regard to the Korean species. Among North American populations, the Oregon ones are monophyletic and sister-group to all of the Eastern samples. Among the Eastern samples, Alabama and Georgia populations are apical sister-groups, except in the case of 1 2 2 and 10 1 1 parameter sets where some North Carolina populations make Georgia samples paraphyletic. In every analysis, North Carolina and Virginia samples are paraphyletic combs which remain quite homogeneous. These results are thus remarkably consistent with the geographical context, the more closely related samples being geographically closer. The tree presented by Kambhampati et al. (1996) differed from our results only in the details of the relationships between Eastern North American samples: geographically close Alabama and Georgia samples were not sister-groups but respectively more related to North Carolina and to Virginia

samples in their results. Additional trees presented by Burnside et al. (1999b) also differed from both studies and had less geographical consistency than ours, since they placed North Carolina as more basal than Virginia samples. In every DNA study, *C. garciai* appears paraphyletic to *C. darwini*.

Sequence divergences between *C. kyebangensis* and the other samples were similar for both genes studied, namely 14.58% for the portion of 12S gene and 14.07% for the portion of 16S gene (Table 2). If this value is used to estimate the age of splitting events with regard to comparable substitution rates most often cited in the literature (concerning mitochondrial genes as COI), the divergence between Asian and North American populations is dated to 18.23–2.13 Myr ago, with most estimates below 10 Myr (*i.e.* during late Miocene) (Table 2). This range is quite broad and difficult to interpret. If one uses the very low rate estimated for the 16S gene of aphid bacteria by Moran et al. (1993)

Table 2. Datations of splitting events between Asian and North American populations according to sequence divergence for the studied fragments of 12S and 16S ribosomal genes, and published substitution rates (sequence divergence corresponds to twice the substitution rate).

Sequence divergence	Substitution rate (percent/Myr)	Age (Myr)
12S		
14.58%	1.1–1.2 (Brower, 1994)	6.08–6.63
—	0.4–0.7 (Andersen et al., 2000)	10.41–18.23
—	2.8–3.3 (Sperling et al., 1997)	2.21–2.60
—	0.02–0.04 (Moran et al., 1993)	182.25–364.50
16S		
14.07%	1.1–1.2 (Brower, 1994)	6.40–8.44
—	0.4–0.7 (Andersen et al., 2000)	10.05–17.59
—	2.8–3.3 (Sperling et al., 1997)	2.13–2.51
—	0.02–0.04 (Moran et al., 1993)	175.88–351.75

Table 3. Datations of splitting events between *Therea petiveriana* and *Cryptocercus* spp. and between Eastern and Western North American populations of *Cryptocercus* according to sequence divergence for the studied fragments of 12S and 16S ribosomal genes, and published substitution rates (sequence divergence corresponds to twice the substitution rate).

Sequence divergence	Substitution rate (percent/Myr)	Age (Myr)
Therea-Cryptocercus		
12S		
21.85%	1.1–1.2 (Brower, 1994)	9.10–9.93
—	0.4–0.7 (Andersen et al., 2000)	15.61–27.31
—	2.8–3.3 (Sperling et al., 1997)	3.31–3.90
—	0.02–0.04 (Moran et al., 1993)	273.13–546.25
16S		
26.65%	1.1–1.2 (Brower, 1994)	11.10–12.11
—	0.4–0.7 (Andersen et al., 2000)	19.04–33.31
—	2.8–3.3 (Sperling et al., 1997)	4.04–4.76
—	0.02–0.04 (Moran et al., 1993)	333.13–666.25
Eastern-Western *Cryptocercus*		
12S		
6.40%	1.1–1.2 (Brower, 1994)	2.67–2.91
—	0.4–0.7 (Andersen et al., 2000)	4.57–8.00
—	2.8–3.3 (Sperling et al., 1997)	0.97–1.14
—	0.02–0.04 (Moran et al., 1993)	80.00–160.00
16S		
8.88%	1.1–1.2 (Brower, 1994)	3.70–4.04
—	0.4–0.7 (Andersen et al., 2000)	6.34–11.10
—	2.8–3.3 (Sperling et al., 1997)	1.35–1.59
—	0.02–0.04 (Moran et al., 1993)	111.00–222.00

and subsequently used by Nalepa et al. (1997) for *Cryptocercus* endosymbiotic bacteria, the date is extremely ancient, ranging between 364.50 and 175.88 Myr, thus between the Carboniferous and Jurassic.

Other splitting events can be tentatively dated the same way, namely the origin of *Cryptocercus* (vicariance between *Therea petiveriana* and *Cryptocercus*) and the vicariance between Eastern and Western North American *Cryptocercus*. Again, both genes give similar sequence divergence values, averaging 23% and 7% respectively (Table 3). This dates *Cryptocercus* origin back to 27.31–3.31 Myr (early Miocene-Pliocene, except if the rate for bacteria from Moran and coworkers are used, dating back to Cambrian- Paleozoic) and North American vicariance to 11.10–0.97 Myr (late Miocene-Pliocene).

CHEMICAL ANALYSIS OF GLANDS

GC profiles revealed the presence of only two major volatile compounds and a pool of components retention times of which correspond to cuticular hydrocarbons. The first peak was identified as linalyl acetate whereas the major peak corresponded to MS spectra of the alcohol 4, 6, 8-trimethyl-7, 9-undecadien-5-ol (Fig. 23).

The two major components identified in the Korean species are part of the female-specific compounds previously identified in females originating from North America (Brossut et al., 1991). However, if we focus on the percentage ratio between the two identified compounds, data showed that the American species from Oregon contained 100% alcohol whereas, in the Virginian species, the glandular secretion was a mixture of about 99% linalyl acetate and 1% alcohol. In the new Korean species, our data revealed the presence of 3% linalyl acetate and about 97% of 4, 6, 8-trimethyl-7, 9-undecadien-5-ol. Therefore, every population studied until now is different, having or not linalyl acetate, and more or less than 4, 6, 8-trimethyl-7, 9-undecadien-5-ol.

DISCUSSION

Cryptocercus kyebangensis appears very similar to North American species from the morphological and anatomical points of view. Even though some characters distinguish it clearly from *C. primarius* or *C. relictus*, the whole aspect of the cockroach remains very similar to its East Asian relatives.

From the molecular point of view, however, *C. kyebangensis* is much more well-differentiated, at the least
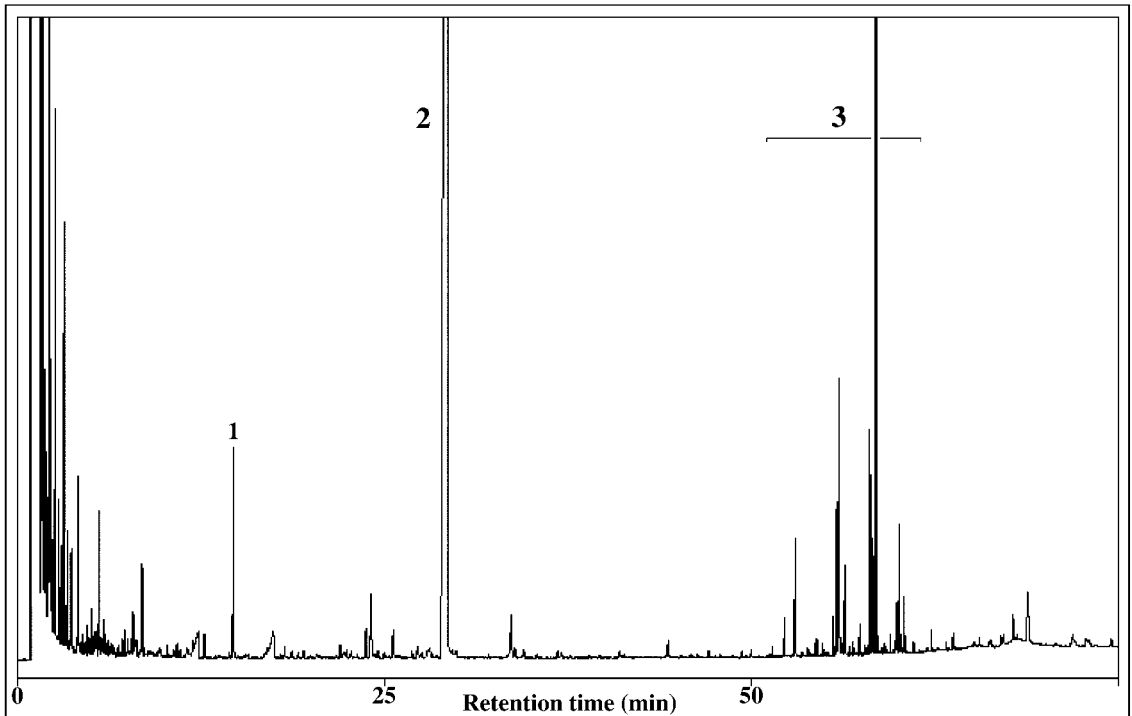
Fig. 23. Typical GC of an extract from female tergite 7 of *Crypocercus kyebangensis*. Peak 1: linalyl acetate; peak 2: 4,6,8-trimethyl-7,9-undecadien-5-ol; peaks 3: unidentified cuticular hydrocarbons. Experimental conditions are given in methods.

from North American taxa. The ribosomal genes as well as the chemical blends of tergal glands are clearly different from those of North American populations belonging to both *C. punctulatus* and *C. clevelandi*. The whole picture of morphological versus molecular differentiations show that a significant—even if rapid—evolution took place after the genus dispersed to North America and split between the Old and the New World. From the phylogenetic point of view, such important changes in ribosomal genes could be responsible for poor results obtained by the phylogenetic analyses carried out until now (for a review, see Grandcolas and D'Haese, 2001). These changes may have decreased the number of informative sites, either increasing autapomorphies of *Cryptocercus* in place of synapomorphies of *Cryptocercus* and related cockroaches, or causing some sites to be saturated and misleadingly synapomorphic to unrelated cockroaches or to termites.

We can now see what is the significance of molecular differences in a historical perspective. The phylogenetic analysis of *Cryptocercus* gave very convincing results since all the obtained cladograms are in total agreement with geographical data and remain so even if the parameters of analysis are changed. A hierarchy of successive vicariances is inferred which fits the geographical hierarchy, from Asia-North America, Eastern-Western North America to locations in Western North America. Even in Eastern North America, phylogenetic relation-

ships agree roughly with a clear NorthEast-SouthWest trend, given that most Northeastern locations (from Virginia to North Carolina) are successively basal (and paraphyletic) to a less Northeastern monophyletic subclade (Alabama and Georgia). Such an agreement with a precise geographical trend was not visible in the tree presented by Kambhampati et al. (1996). We can hypothesize that the incorporation of one East Asian *Cryptocercus* and Polyphagid relatives (*Therea petiveriana*) in the data set has improved both homology and polarization in the analysis so that tree topology becomes more accurate.

In this framework, *C. kyebangensis* is the sister-group to North American *Cryptocercus*, as a representative of an East Asian sub-clade having differentiated earlier than the different North American sub-clades from their common North American ancestor. This result corroborates the biogeographical scenario beginning with the North America-East Asia vicariance (Bey-Bienko, 1950; Grandcolas, 1994b, 1999b, 1999c; Nalepa et al., 1997).

It is also worthwhile to consider the amount of sequence divergence between different *Cryptocercus* taxa.

First, the level of sequence divergence is very similar between the two genes at each level considered, a point that strengthens the conclusions drawn from these divergence values (Tables 2 and 3). In addition, the variance between different taxa within each vicariance level

(North America versus East Asia, etc.) is also weak (much lower than 5%), and allows us to compute reasonable mean values of divergence between non-monotypic sister-groups. Clearly, divergence is much more important (about 2.5 times more) between East Asian and North American taxa than among North American taxa. Even the divergence between *Therea petiveriana* and *C. kyebangensis* which are very differentiated from a morphological point of view is comparatively not much higher (about 1.5 times more than between East Asian and North American taxa). This shows how rapidly some molecular characteristics of *Cryptocercus* could have evolved even if morphology remains very stable as a whole.

Second, most measures of sequence divergence in the literature used the concept of molecular clock to obtain datations. These dates can be very inaccurate because of methodological problems associated with the molecular clock, beginning with frequent lack of paleontological calibration and ending with highly variable substitution rates found in different genes for different groups (see Grandcolas, 1999b for discussion in the context of *Cryptocercus*). We have chosen a very careful and conservative treatment of the subject aimed at providing basic and refutable datation hypotheses. We consider these hypotheses as obviously speculative and do not give them much credit.

Another reason for the speculative value of previous datations in the case of *Cryptocercus* is that several studies have conflated datations obtained from bacteria symbionts and datations of cockroach clades (*e.g.,* Bandi et al., 1995; Nalepa et al., 1997). Even if cospeciation can be an expected result, it has first to be carefully demonstrated before to use symbiont rates in places of rates obtained from cockroaches themselves.

According to diverse substitution rates cited in the literature concerning mitochondrial genes, we estimated dates for splitting events between *Therea-Cryptocercus*, *C. kyebangensis* and North American *Cryptocercus* and between Eastern and Western North American *Cryptocercus* (Tables 2 and 3). These dates are very broad, especially with respect to Moran et al. (1993) estimate of rates (for bacteria symbionts of insects) which completely depart from the others (for insects) cited in literature. We are inclined to consider that these slow rates cannot reasonably apply here, given that they would date the North American splitting of *Cryptocercus* to the Carboniferous and the older splitting events (between *Therea* and *Cryptocercus*) to before the Cambrian. In addition, Maekawa and Matsumoto (2000) made an attempt of dating using a molecular clock but using COII sequences from *C. punctulatus* and *C. relictus*. The sequence divergence was 14.5% (uncorrected pairwise difference), thus extremely close to the present one inferred from the 12S and 16S ribosomal genes (Table 1), but the authors also used an especially low substitution rate (from Beckenbach et al., 1993)

among diverse ones available for mitochondrial genes including COII. Using this rate, they dated the beringian vicariance back to the Eocene-Oligocene. Considering the other substitution rates which are altogether congruent, we obtain more recent datations. The origin of *Cryptocercus* could have been comprised in the Miocene-Pliocene, the beringian vicariance also in the Miocene-Pliocene and the North American splitting in the late Miocene to early Pleistocene. These dates are totally consistent with independent cladistic biogeographic estimates (Grandcolas, 1994b, 1999b, 1999c) which used, among other paleogeographical cues, the changes in the position of India, the area of endemism of the sister-group to *Cryptocercus*, to date the origin of *Cryptocercus*. These dates can be contrasted with those mentioned by Nalepa et al. (1997) with endosymbiotic bacteria which mentioned a range of 70–25 Myr for the North American splitting (thus Palaeocene to Oligocene). This range was obtained using 16rDNA sequences of bacteria (also Bandi et al., 1995) and the substitution rates mentioned by Moran et al. (1993) for bacteria. Besides the discrepancy related to the use of a strikingly low rate of substitution, it is worth noting that the sequence divergence between North American *Cryptocercus* was 1.85% (corrected for multiple substitution according to the model of Kimura, 1980), thus much lower than the two coherent estimates obtained here from two genes of cockroaches, ranging from 6.40 to 8.88% (Table 3). Using this divergence estimate with lower and roughly mutually consistent substitution rates, we would obtain a recent date for the North American splitting of *Cryptocercus*, ranging 2.31–0.28 Myr (early Pliocene-Pleistocene).

These recent Late Cenozoic dates for all these events are also consistent with recent molecular estimates for beringian vicariances in several plant groups (Xiang et al., 2000), ranging 12.56–0.31 Myr with most estimates during the late Miocene and Pliocene. This is also consistent with other dates of these vicariances, obtained from either molecular or paleontological data (Wen, 1999).

Concerning tergal glands, the results demonstrated that the studied populations of *Cryptocercus* differ in their chemical blends, a fact already established from North American populations (Brossut et al., 1991). For the record, these blends did not vary according to age or reproductive status in North American populations (Brossut et al., 1991). *C. kyebangensis* is shown here to be different again in this respect from the studied populations in North America. Such variation may not be uncommon in insect pheromones and have already been reported in Heteroptera (Aldrich et al., 1989, 1993; Brézot et al., 1994), Coleoptera (Lanier et al., 1980) and Lepidoptera (Haynes and Hunt, 1990; Löfstedt, 1990). These results would benefit from comparison with the analyses of products of the similar

tergal glands in other Polyphaginae cockroaches (Brossut and Roth, 1977; personal observation).

The description of *C. kyebangensis* puts in a new perspective the knowledge obtained about *Cryptocercus* which mainly deals with North American species. This perspective is diverse, showing both uniform and contrasted characteristics at the genus level. From the morphological and anatomical points of views, *C. kyebangensis* is very similar to all other described species. From the molecular point of view, *C. kyebangensis* appears to differ markedly from North American populations, indicating that a very significant amount of evolution has occurred since the beringian vicariance occurred. It will be interesting to document whether this evolutionary divergence also concerns the key features of *Cryptocercus*, namely those related to feeding behavior, social behavior and symbiosis. Future studies (Park and Choe, in prep.) will bring additional information in this regard. Has the social behavior, diet and symbiosis of *C. kyebangensis*, even if they are mostly comparable to those of *C. punctulatus*, evolved distinctively in many details?

In conclusion, the misleading "primitive taxon" concept has often been applied to *Cryptocercus*. The whole picture about this East Asian species claims for heterobathmy in the framework of the study of the amphiberingian genus *Cryptocercus*. Different character systems evolving at strikingly different rates clearly show how this concept cannot be used presently.

ACKNOWLEDGMENTS

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APPENDIX 1

Alignment of mitochondrial 12S rRNA gene sequences according to the parameter set 2 1 1 of the program POY. North American populations of *Cryptocercus clevelandi*, *C. punctalatus*, *C. wrighti*, *C. darwini*, and *C. garciai* are indicated by the name of the respective localities. (See Fig. 22.)

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                   1 1111111112 2222222223 3333333334 4444444445 5555555556
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Blatta orientalis TACTATGTTA CGACTTATCT A--A-ACTAA TAATTAATGA GAGTGACGGG CGATGTGTAC
Therea petiveriana .......... .......... C--.-C..-- .-.A...C.. ...C...... ..........
C. kyebangensis   .......... .......... C------G.. ...A...C.. ...C...... ..........
Cave junction     .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
FSR-4611          .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
Glendale          .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
Selma             .......... .......... CTT.TTT... ...A...C.. ...C...... A...A.....
Union Creek       .......... .......... CTT.TTT... ...A...C.. ...C...... A...A.....
Dripping Rock     .......... .......... CTT.TTT... ...A...C.. ...C...... ....A...T.
Mtn. Lake         .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
Powell Gap        .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
Asheville         .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
Highlands         .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
Swain Co.         .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
B.R. Mtn.         .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
Cherry Log        .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
Cheaha St. Park   .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
Lk. Guntersville  .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
L.R. Canyon       .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....
Mentone           .......... .......... CTT.TTT... ...A...C.. ...C...... ....A.....


                                                          1 1111111111 1111111111
          6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
          1234567890 1234567890 1234567890 1234567890 1234567890 1234567890

Blatta orientalis ATATTCTAGA GCCAAATGCA TTTTTACAAT CT-ACA-AAA AATTACAATT AAATCCAATT
Therea petiveriana .......... ..T...AT.. CC.CA...T. ..-.A.-TC. ......T... .......CC.
C. kyebangensis   .C........ AA.T..AT.. .CAA...... ..A.A.T... .......T.C ..........
Cave junction     .C.C..C... AA.TT.GT.. ..AA...T.. ..A.A.T..T .......T.C ..........
FSR-4611          .C.C..C... AA.TT.GT.. ..AA...T.. ..A.A.T..T .......T.C ..........
Glendale          .C.C..C... AA.TT.AT.. ..AA...T.. ..A.A.T..T .......T.C ..........
Selma             .C.C..C... AA.TT.AT.. ..AA...T.. ..A.A.T..T .......T.C ..........
Union Creek       .C.C..C... AA.TT.AT.. ..AA...T.. ..A.A.T..T .......T.C ..........
Dripping Rock     .C....C... CA.TT.AT.. ..A....T.. ..A.A.T..T .......T.C ..........
Mtn. Lake         .C....C... AA.TT.GT.. .AA....T.. ..A.A.T..T .......C.C ..........
Powell Gap        .C....C... AA.TT.AT.. .AA....T.. ..C.ACT..T .......T.C ..........
Asheville         .C...TA... AA.TT.AT.. CAA....T.. ..A.A.T..C .......T.C ..........
Highlands         .C...TA... AA.TT.AT.. .AA....T.. ..A.A.T..T .......T.C ..........
Swain Co.         .C...TA... AA.TT.AT.. .AA....T.. ..A.A.T..T .......T.C ..........
B.R. Mtn.         .C...TA.A. AA.TT.AT.. .AA....T.. ..A.A.T..C ..A....T.C ..........
Cherry Log        .C...TA... AA.TT.AT.. .AA....T.. ..A.A.T..T .......T.C ..........
Cheaha St. Park   .C...TA... AA.TT.AT.. AAA....T.. ..A.A.T..T .......T.C ..........
Lk. Guntersville  .C...TA... AA.TT.AT.. .AA....T.. ..A.A.T..T .......T.C ..........
L.R. Canyon       .C...TA... AA.TT.AT.. .AA....T.. ..A.A.T..T .......T.C ..........
Mentone           .C...TA... AA.TT.AT.. AAA....T.. ..A.A.T..T .......T.C ..........


          1111111111 1111111111 1111111111 1111111111 1111111111 1111111111
          2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
          1234567890 1234567890 1234567890 1234567890 1234567890 1234567890

Blatta orientalis TAATAAAAAA ATTTCAATTA ATAATCCAAT AATTAATATA ---AATGTAA TCCATCTCCA
Therea petiveriana .C..TT...T ...A...C.T .A.T....T. .TGCCC.... CGC.T..... .........T
C. kyebangensis   .C...TT... ......TC.. .A.......A ...A....A. ---..A.... C....T..T.
Cave junction     .C...TT... ...A....C .....T...A C..A....AC ---....... C....AAT..
FSR-4611          .C...TT... ...A....C .....T...A C..A....AC ---....... C....AAT..
Glendale          .C...TT... ...A....C .C...T...A C..A....AC ---....... C....AAT..
Selma             .C...TT... ...A....C .C...T...A C..A....AC ---....... C....AAT..
Union Creek       .C...TT... ...A....C .C...T...A C..A....AC ---....... C....AAT..
Dripping Rock     .C...TT... ...A..... .C...T...G C..A....AC ---....... C....AAT..
Mtn. Lake         .C...TT... ...A..... .....T...G C..A....AC ---....... C....AAT..
Powell Gap        .C...TT... ...A..... .C...T...G C..A....AC ---....... C....AAT..
Asheville         .C...TC... ...A....C .C...T...A C..A....AT ---....... C....AA...
Highlands         .C...TT... ...A..... .........G C..A....AC ---....... C....AAT..
Swain Co.         .C...TT... ...A..... .........G C..A....AC ---....... C....AAT..
B.R. Mtn.         .C...TT... ...A..... .........A C..A....AC ---....C. C....AAT..
Cherry Log        .C...TT... ...A..... .........G C..A.C.AC ---....... C....AAT..
Cheaha St. Park   .C...TT... ...A..... .........G C..A....AC ---....... C....AAT..
Lk. Guntersville  .C...TT... ...A..... .........G C..A....AC ---....... C....AAT..
L.R. Canyon       .C...TT... ...A....C .........G C..A....AC ---....... C....AAT..
Mentone           .C...TT... ...A...... .........G C..A....AC ---....... C....AAT..
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Blatta orientalis   CCAAATCATA AACTGCACCT TGACCTGAAA TAATATTTTA AAATAAATCA AGAAAATTAA
Therea petiveriana  .TT....... ..T.AA.... .......... ...AT.CAA. T..A...A-. .....T..T.
C. kyebangensis     .TT....... ..T....... .......... ....T.AAA. T..A...A.C ..........
Cave junction       .TT..CT... ..T....... .......... ...AT.AAA. T..A...A.. ........TT
FSR-4611            .TT..CT... ..T....... .......... ...AT.AAA. T..A...A.. ........TT
Glendale            .TT..CT... ..T....... .......... ...AT.AAA. T..A...A.. ........TT
Selma               .TT..CT... ..T....... .......... ...AT.AAA. T..A...A.. ........TT
Union Creek         .TT..CT... ..T....... .......... ...AT.AAA. T..A...A.. ........TT
Dripping Rock       .TT..CT... ..T......A .......... ...A..AAA. T..A..A... ..........
Mtn. Lake           .TT..CT... .........A .......... ...A..AAA. T..A...A.. ..........
Powell Gap          .TT..CT... ..T......A .......... ...A..AAA. T..A...A.. ..........
Asheville           .TT....... ..T....... .......... ...A..AAA. T..A...A.. ..........
Highlands           .TT...T... ..T......A .......... ...A..AAA. T..A...A.. ..........
Swain Co.           .TT...T... ..T......A .......... ..G...AAA. T..A...... ..........
B.R. Mtn.           .TT..CT... ..T......A .......... ......AAA. T..A...A.. ..........
Cherry Log          .TT..CT... ..T......A .......... ......AAA. T..A...A.. ..........
Cheaha St. Park     .TT...T... ..T......A .......... ......AAA. T..A...... ..........
Lk. Guntersville    .TT...T... ..T......A .......... ......AAA. T..A...... ..........
L.R. Canyon         .TT...T... ..T......A .......... ......AAA. T..A...... ..........
Mentone             .TT...T... ..T......A .......... ......AAA. T..A...... ..........


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Blatta orientalis   TAA-ATCTCT AAAAAGATCT TCATTATAAC GGCGGTATAC ATA----CA- ATAAA-TTTG
Therea petiveriana  -CT-.-.C.. .......CAC ..TAC.A... A........T .A.-----.- TC.G.-..AA
C. kyebangensis     -.--.-.A.. .C.......C ..A..C.... .......... .A.T---.-- .....-..AA
Cave junction       -.T-.-.T.. .....A.CTC ...AA.C... .......... .A.TAAAATC T...G-.CAA
FSR-4611            -.T-.-.T.. .....A.CTC ...AA.C... .......... .A.TAAAATC T...G-.CAA
Glendale            -.T-.-.T.. .....A.CTC ...AA.C... .......... .A.TAAAATC T...G-.CAA
Selma               -.T-.-.T.. .....A.CTC ...AA.C... .......... .A.TAAAATC T...G-.CAA
Union Creek         -.T-.-.T.. .....A.CTC ...AA.C... .......... .A.TAAAATC T...G-.CAA
Dripping Rock       -.TA.-.... .......CT. ...AA.C... .......... .A.TAAATTC ......-.CAA
Mtn. Lake           -.TA.-.... .......CT. ...AA.C... .......... .A.TAAGT.T ......-.CAA
Powell Gap          -.TA.-.... .......CT. ...AA.C... .......... .A.TAAATTC ......-.CAA
Asheville           -.CA.-.... .......CT. ...AAGC... .......... .A.TAAAT.T .A...-.CAA
Highlands           -.CA.-.... .......CT. ...AA.C... .......... .A.TAAAT.T ......-.CAA
Swain Co.           -TCA.-.... .......CT. ...AA.C... .......... .A.TAAAT.T TC.A..-.CAA
B.R. Mtn.           -TCA.-.... .......CTC ...AA.C... .......... .A.TAAAT.T ......-.CAA
Cherry Log          -TCA.-.... .......CT. ...AA.C... .......... .A.TAAAT.T ......-.CAA
Cheaha St. Park     -TCA.-.... .......CT. ...AA.C... .......... .A.T-AAT.T .....T.AAA
Lk. Guntersville    -TCA.-.... .......CT. ...AA.C... .......... .A.T-AAT.T .....T.AAA
L.R. Canyon         -TCA.-.... .......CT. ...AA.C... .......... .A.T-AAT.T .....T.AAA
Mentone             -TCA.-.... .......CT. ...AA.C... .T........ .A.T-AAT.T .....T.AAA


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Blatta orientalis   GTAAG-GTTC AACGCGGATT ATCAATTAAG GGACAGATTC CTCTAAATAG ACTATAATAC
Therea petiveriana  .....TA... ....T...A. ...G....T. .......... .......GA.. ....A.....
C. kyebangensis     .....-..C. ....A..... ...G..A..A .A....G... ....G..C.. .A..A.....
Cave junction       .....-..C. .......... ...G..A..A AT....G... ....G..C.. .A..A.....
FSR-4611            .....-..C. .......... ...G..A..A AT....G... ....G..C.. .A..A.....
Glendale            .....-..C. .......... ...G..A..A AT....G... ....G..C.. .A..A.....
Selma               .....-..C. G......... ...G..A..A AT....G... ....G..C.. GA..A.....
Union Creek         .....-..C. .......... ...G..A..A AT....G... ....G..C.. .A..A.....
Dripping Rock       .....-..C. .......... ...G..A..A AT....G... ....G..A.. .A..A.....
Mtn. Lake           .....-..C. ....G..... ...G..A..A AT....G... ....G..A.. .A..A.....
Powell Gap          .....-..C. .......... ...G..A..A AT....G... ....G..A.. .A..A.....
Asheville           .....-..C. ....A..... ...G..A.CA AC....G... ....G..... .A..A.....
Highlands           .....-..C. ....A..... ...G..A.CA AC....G... ....G..G. .TA.A.....
Swain Co.           .....-..C. ....A..... ...G..A.CA AC....G... ....G..... .A..A.....
B.R. Mtn.           .....-..C. ....A..... ...G..A.CA AC....G... ....G..... .A..A.....
Cherry Log          .....-..C. ....A..... ...G..A.CA AC....G... ....G..... .A..A.....
Cheaha St. Park     .....-..C. ....A..... ...G..A.CA AC....G... ....G..... .A..A.....
Lk. Guntersville    .....-..C. ....A..... ...G..A.CA AC....G... ....G..... .A..A.....
L.R. Canyon         .....-..C. ....A..... ...G..A.CA AC....G... ....G..... .A..A.....
Mentone             .....-..C. ....A..... ...G..A.CA AC....G... ....G..... .A..A.....
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Blatta orientalis       CGCCAAATTC TTTAAGTTTC AAGACCTT-A -ACTAGTACT ACCTAAGTTA AAATAATTTA
Therea petiveriana      .......... .......... ....T.A.A. T....A.... ..TC.G..A- C..A.C....
C. kyebangensis         T......... .......... ....T.A.-. -T...T.... ..TC.G...- ........A.
Cave junction           T......... ...G...... G...AAA.-G -.AC.T.... ..TC.....- ..........
FSR-4611                T......... ...G...... G...AAA.-G -.AC.T.... ..TC.....- ..........
Glendale                T......... ...G...... G...AAA.-G -.AC.T.... ..TC.....- ..........
Selma                   T......... ...G....C. G...AAA.-G -..C.T.... ..TC.....- ..........
Union Creek             T......... ...G...... ....AAA.-G -.AC.T.... ..TC.....- ..........
Dripping Rock           T......... ...G...... ....AAA.-. -..A.T.... ..TC.....- ....-...A.
Mtn. Lake               T......... ...G...... ....AAA.-. -..A.T.... ..TC.....- ....-...A.
Powell Gap              T......... ...G...... ....AAA.-. -..A.T.... ..TC.....- ....-...A.
Asheville               T......... ...G...... ....AAA.-. -..A.T.... ..TC.....- ....-...A.
Highlands               T......... ...G...... ....AAA.-. -..A.T.... ..TC.....- ....-...A.
Swain Co.               T......... ...G...... ....AAA.-. -..A.T.... ..TC.....- ....-...A.
B.R. Mtn.               T......... ...G...... ....AAA.-. -..A.T.... ..TC.....- ....-...A.
Cherry Log              T......... ...G...... ....AAA.-. -..A.T.... ..TC.....- ....-...A.
Cheaha St. Park         T......... ...G...... ....AAA.-. -..A.T.... ..TC.....- ....-...A.
Lk. Guntersville        T......... ...G...... ....AAA.-. -..A.T.... ..TC.....- ....-...A.
L.R. Canyon             T......... ...G...... ....AAA.-. -..ATT.... .ATC.....- ....-...A.
Mentone                 T......... ...G...... ....AAA.-. -..A.T.... ..TC.....- ....-...A.


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Blatta orientalis       TATCT-AAAT ATTAGGGTAT CTAATCCTAG TTT
Therea petiveriana      C...AA.... .A........ .......... ...
C. kyebangensis         C....A.... .A........ .......... ...
Cave junction           C..AAA.... -A.-...... .......... ...
FSR-4611                C..AAA.... -A.-...... .......... ...
Glendale                C..AAA.... -A.-...... .......... ...
Selma                   C..AAA.... -A.-...... .......... ...
Union Creek             C...AA.... -A.-...... .......... ...
Dripping Rock           C..AAA.... -A.-...... .......... ...
Mtn. Lake               C..AAA.... -A.-...... .......... ...
Powell Gap              C..AAA.... -A.-...... .......... ...
Asheville               C..AAA.... -A.-...... .......... ...
Highlands               C..AAA.... -A.-...... .......... ...
Swain Co.               C.CAAA.... -A.-...... .......... ...
B.R. Mtn.               C..AAA.... -A.-...... .......... ...
Cherry Log              C..AAA.... -A.-...... .......... ...
Cheaha St. Park         C..AAA.... -A.-...... .......... ...
Lk. Guntersville        C..AAA.... -A.-...... .......... ...
L.R. Canyon             C..AAA.... -A.-...... .......... ...
Mentone                 C..AAA.... -A.-...... .......... ...
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APPENDIX 2

Alignment of mitochondrial 16S rRNA gene sequences according to the parameter set 2 1 1 of the program POY, North American populations of *Cryptocercus clevelandi, C. punctulatus, C. wrighti, C. darwini,* and *C. garciai* are indicated by the name of the respective localities. (See Fig. 22.)

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Blatta orientalis        TTACGCTGTT ATCCCTAAGG TAATTTAATC TCATAATCAA CAAAAATTGG ATCAAAAAAA
Therea petiveriana       .......... ......T... .......G.. .T.......C ...T..-... ...TTT....
C. kyebangensis          .......... ......T... .......G.. .T....... ......-... ......T.T.
Cave junction            .......... .......... .......C.. .T...G...C T.....-... ........T.
FSR-4611                 .......... .......... .......C.. .T...G...C T.....-... ........T.
Glendale                 .......... .......... .......C.. .T...G...C T.....-... ........T.
Selma                    .......... .......... .......C.. .T...G...C T.....-... ........T.
Union Creek              .......... .......... .......C.. .T...G...C T.....-... ........T.
Dripping Rock            .......... .......... .......G.. .G.......C T.....-... ........TT
Mtn. Lake                .......... .......... .......G.. .T...G...C T.....-... ........TT
Powell Gap               .......... .......... .......G.. .T.......C T.....-... ........TT
Asheville                .......... .......... .......G.. .T......T T.....-... ........CT
Highlands                .......... .......... .......G.. .T......T T.....-... ........CT
Swain Co.                .......... .......... .......G.. .T......T T...G.-... ........CT
B.R. Mtn.                .......... .......... .......G.. .T......T T.....-... ........TT
Cherry Log               .......... .......... .......G.. .T......T T.....-... ........TT
Cheaha St. Park          .......... .......... .......C.. .T...G...C T.....-... ........CT
Lk. Guntersville         .......... .......... .......C.. .T...G...C T.....-... ........CT
L.R. Canyon              .......... .......... .......G.. .T......T T.....-... ........CT
Mentone                  .......... .......... ......GC. .T......T T.....-... ........CT


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Blatta orientalis        TATAAATTAA TAT---ATAA ATAAAAAAAA GAGTTTTTTT ATTTCTTCCT ATCACCCCAA
Therea petiveriana       .TC.T.AC.. ..A---CAGT .A...C.T.C .....A.... T-A...CG.. ....T.....
C. kyebangensis          .......A.. ...---.-.. .A..T..... .....AA..A .-A....T.C ..........
Cave junction            .......A.. ...---.-.. .A..T..... .....A.... .-A....... ..........
FSR-4611                 .......A.. ...---.-.. .A..T..... .....A.... .-A....... ..........
Glendale                 .......A.. ...---.-.. .A..T..... .....A.... .-A....... ..........
Selma                    .......A.. ...---.-.. .A..T..... .....A.... .-A....... ..........
Union Creek              .......A.. ...---.-.. .A..T..... .....A.... .-A....... ..........
Dripping Rock            ......CA.. ...---.-.. .A..T..... .....A.A. .-A......C ..........
Mtn. Lake                .......A.. ...---..-T. .A..T..... .....A.A. .-A......C ..........
Powell Gap               ......CA.. ...---.-.. .A..T..... .....A.A. .-A......C ..........
Asheville                .......A.. ...--.-.. .A..T..... .....A.A. .-A......C ..........
Highlands                .......A.. ...--AA.-.. .A..T..... .....A.A. .-A......C ..........
Swain Co.                .......A.. ....--A.-.. .A..T..... .....A.A. .-A......C ..........
B.R. Mtn.                ......CA.. ...-AA.-.. .A..T..... .....A.A. .-A......C ..........
Cherry Log               ......CA.. ...--A.-.. .A..T..... .....A.AC. .-A......C ..........
Cheaha St. Park          .......A.. ....--A.-.. .A..T..... .....A.A. .-A......C ..........
Lk. Guntersville         .......A.. ...-AA.-.. .A.TT..... .....A.A. .-A......C ..........
L.R. Canyon              .......A.. ...AAA.-.. .A.TT..... .....A.A. .-A......C ..........
Mentone                  .......A.. ...AAA.-.. .A.TT..... .....A.A. .-A......C ..........


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                          1234567890 1234567890 1234567890 1234567890 1234567890 1234567890

Blatta orientalis        CAAAATACTT TTAAATA-TT AAATTATATC AAACACTAA- -TAAATAATT --TAAAAATA
Therea petiveriana       .......TAA ..CCCAG-.. ..T.A.-CC. TC.TT....C CA..C..... ACA...T...
C. kyebangensis          .......AA. ..TC.A.-.. ..AA.-..A TC.T..-..- -A...--..A --A...T...
Cave junction            ......TTA- CAC..C.-.. .....--T.T .CC...-..- -A....-... --....T.A.
FSR-4611                 ......TTA- CAC..C.-.. .....--T.T .CC...-..- -A....-... --....T.A.
Glendale                 ......TTA- CAC..C.-.. .....--T.T .CC...-..- -A....-... --....T.A.
Selma                    ......TTA- CAC..C.-.. .....--T.G .CC...-..- -A....-... --....T.A.
Union Creek              ......TTA- CAC..C.-.. .....--T.G .CC...-..- -A....-... --....T.A.
Dripping Rock            ......TTA- CAT....C.A ..CA..-T.A .CC...-..- -A.....-..G --A..TT.A.
Mtn. Lake                ......TTA- CAT....-.. ......-T.A .CC...-..- -A.....-..G --A..TT.A.
Powell Gap               ......TTA- CAT....C.A ..CA..-T.A .CC...-..- -A.....-..G --A..TT.A.
Asheville                ......T.A- AAT....C.A ...A.C-T.A TCC...-..- -A.....--AA --A..TCGA.
Highlands                ......TTA- AAT....T.A ......-T.A TCC...-..- -A.....AA --A..TT.A.
Swain Co.                ......TTA- AAT....T.A ......-T.A TCC...-..- -A.....AA --A..TT.A.
B.R. Mtn.                ......TTA- AAT....T.A ......-T.A TCC...-..- -A.....AA --A..TC.A.
Cherry Log               ......T.A- AAT....T.A ......-T.A TCC...-..- -A.....AA --A..TT.A.
Cheaha St. Park          ......TTA- AAT....T.A ......-T.A TCC...-..- -A.....AA --A..TT.A.
Lk. Guntersville         ......T.A- AAT....T.A ......-T.A TCC...-..- -A.....AA --A..TT.A.
L.R. Canyon              ......TTA- AAT....T.A ......-T.A TCC...-..- -A.....AA --A..TT.A.
Mentone                  ......TTA- AAT....T.A ......-T.A TCC...-..- -A.....AA --A..TT.A.
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Blatta orientalis  T-TAAACTCT ATAGGGTCTT CTCGTCCCAC AAAAAAACTT AAGCATTTTT ACTTAAATTT
Therea petiveriana .-........ .......... .........T G....C.T.. .GA.-C.... .TC....AA.
C. kyebangensis    .A........ .......... .........A .....T.T.. .G.......A ..C....A..
Cave junction      .-C....... .......... .........A .....T.T.. .G.......A ..C....A..
FSR-4611           .-C....... .......... .........A .....T.T.. .G.......A ..C....A..
Glendale           .-........ .......... .........A .....T.T.. .G.......A ..C....A..
Selma              .-........ .......... .........A .....T.T.. .G.......A ..C....A..
Union Creek        .-........ .......... .........A .....T.T.. .G.......A ..C....A..
Dripping Rock      .-........ .......... .........A .....T.T.C .G.......A ..C...TA..
Mtn. Lake          .-C....... .......... .........A .....T.T.. .G.......A ..C....A..
Powell Gap         .-........ .......... .........A .....T.T.C .G.......A ..C...TA..
Asheville          .-........ .......... .......... .....T.T.. .G.......A ..C....A..
Highlands          .-........ .......... .........A ....TT.T.. .G.......A ..C....A..
Swain Co.          .-........ .......... .........A ....TT.T.. .G.......A ..C....A..
B.R. Mtn.          .-........ .......... .........A .....T.T.. .G.......A ..C....A..
Cherry Log         .-........ .......... .........A .....T.T.. .G.......A ..C....A..
Cheaha St. Park    .-........ .......... .........A ....TT.T.. .G.......A ..C....A..
Lk. Guntersville   .-........ .......... .........A ....TT.T.. .G.......A ..C....A..
L.R. Canyon        .-........ .......... .........A ....TT.T.. .G.......A ..C....A..
Mentone            .-........ .......... .........A ....TT.T.. .G.......A ..C....A..


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                   4444444445 5555555556 6666666667 7777777778 8888888889 9999999990
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Blatta orientalis  CAAGTTC-A- ATAA----AA TTTTAAAGAA ACAGTATATT CCTCGTCCAA CCATTCATTC
Therea petiveriana T..A...--- -...----T. .A..T.T-.C TA..-.C.G. -..-.-.ACT T.G.C..-A.
C. kyebangensis    T..A...-.- ....----.T AAA.T..-.G .....T..CA .......A.. ..........
Cave junction      T..A...A.C ....T---.. AAA.C..-.G ......AT.A .......A.. ..........
FSR-4611           T..A...A.C ....T---.. AAA.C..-.G ......AT.A .......A.. ..........
Glendale           T..A...A.C ....T---.. AAA.C..-.G ......AT.A .......A.. ..........
Selma              T..A...A.C ...GT---.. AAA.C..-.G ......GT.A .......A.. ..........
Union Creek        T..A...A.C ....T---.. AAA.C..-.G ......GT.A .......A.. ..........
Dripping Rock      T..A...A.C .A..T---.. AAA.TT.-.G .A....A.CA .......A.. ........C.
Mtn. Lake          T.....A.A .A..T---.. AAA.TT.-.G ......A..A ...T..A.. T.........
Powell Gap         T..A...A.C .A..T---.. AAA.TT.-.G .A....A.CA .......A.. ........C.
Asheville          T..A...A.C .A..T---.. AAA.TT.-.G .A....A..A ..T..A. .A.
Highlands          T..A...A.C .A..T---.. AAA.TT.-.G .A.......A .......A.. ..........
Swain Co.          T..A...A.C .A..T---.. AAA.TT.-.G .A....A..A .......A.G ..........
B.R. Mtn.          T..A...A.C .A..T----. AAA..T.-.. .T....A..A .......A.. ..........
Cherry Log         T..A...A.T .A..TAAA.. AAA.TT.-.. .....T..A .......A.G ..........
Cheaha St. Park    T..A...A.C .A..T---.. AAA.TT.-.G .A....A..A .......A.. ..........
Lk. Guntersville   T..A...A.C .A..T---.. AAA.CT.-.G .A....A..A .......A.. ..........
L.R. Canyon        T..A...A.C .A..T---.. AAA.CT.-.G .A....A..A .......A.. ..........
Mentone            T..A...A.C .A..T---.. AAA.TT.-.G .A....A.CA .......A.. ........C.


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Blatta orientalis  CAGTCCACAA TTAAAAGGAC TAATGATTAT GCTACCTTTG CACGGTCAAA ATACCGCGGC
Therea petiveriana T.-.-T.... ....T.-.... CTC.....C .......... .......G.. G.G....A.A
C. kyebangensis    ...C.T.... ......-.... .......... .......... .-........ ..........
Cave junction      ...C.T.... ......-.... .......... .......... .......G.. ..........
FSR-4611           ...C.T.... ......-.... .......... .......... .......... ..........
Glendale           ...C.T.... ......-.... .......... .......... .......... ..........
Selma              ...C.T.... ......-.... .......... .......... .......... ..........
Union Creek        ...C.T.... ......-.... .......... .......... .......... ..........
Dripping Rock      ...CATT... ......-A.. .......... .......... ...A...... ....T.....
Mtn. Lake          ...CATT... ......-A.. .......... .......... ...A...... ....T.....
Powell Gap         ...CATT... ......-A.. .......... .......... ...A...... ....T.....
Asheville          A..CATT... ......-A.. .......... .......... ...A...... ....T.....
Highlands          ...CATT... ......-A.. .......... .......... ...A...... ....T.....
Swain Co.          ...CATT... ......-A.. .......... .......... ...A...... ....T.....
B.R. Mtn.          ...CATT... ......-T.. .......... .......... ...A...... ....T.....
Cherry Log         ...CATT... ......-A.. .......... .......... .G.A...... ....T.....
Cheaha St. Park    ...CATT... ......-A.. .......... .......... ...A...... ....T.....
Lk. Guntersville   ...CATT... ......-A.. .......... .......... ...A...... ....T.....
L.R. Canyon        ...CATT... ......-A.. .......... .......... ...A...... ....T.....
Mentone            ...CATT... ......-A.. .......... .......... ...A...... ....T.....
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                          3333333333 3333333333 3333333333 3333333334 4444444444 4444444444
                          6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
                          1234567890 1234567890 1234567890 1234567890 1234567890 1234567890

Blatta orientalis        CC-TTCAAAT AAATTTCAGT GGGCAGGTTA TACTTCATAA AT-AAATT-- ACAGAAGAGA
Therea petiveriana       AA-.....C. -T.--...A. .......CC. .T.C..C-.G T.C.C.AC-- .T......A.
C. kyebangensis          ..-..-C... -.C--..... .......GC. ...A.-.-.. ..-.T...-- CA........
Cave junction            .T-.C-.... -..--....C ........C. ...A...-T. ..--T.CA-- G..A......
FSR-4611                 .T-.C-.... -..--....C ........C. ...A...-T. ..--T.CA-- G..A......
Glendale                 .T-.C-.... -..--....C ........C. ...A...-T. ..--..CA-- G..A......
Seima                    .T-.C-.... -..--....C ........C. ...A...-T. ..--..CA-- G..A......
Union Creek              .T-.C-.... -..--....C ........C. ...A...-T. ..--..CA-- G..A......
Dripping Rock            .A-..-T..A -..--..... .......A. ...A...-.. ..-.T..A-- CA........
Mtn. Lake                .A-..-T..A -..--..... ......A.AC ...A...-.. ..-.T..A-- CA........
Powell Gap               .A-..-T..A -..--..... .......A. .......-T. ..-.T..A-- CA........
Asheville                .A-..-TT.A -..--..... ......CA. ......--.. ..-.....A-- CA........
Highlands                .A-..-T..A -..--..... ......CA. ......-.. ..-.....A-- CA........
Swain Co.                .A-..-T..A -..--..... ......CA. ......--.. ..-.....-A CA........
B.R. Mtn.                .AT..-T..A -..--..... T......CA. ......--.. ..-.....-- CA........
Cherry Log               .AT..-T..A -..--..... T......CA. ......--.. ..-.....-- CA........
Cheaha St. Park          .AT..-..A -..--..... .......C-G .......--.G TA-..T..-- TAT.......
Lk. Guntersville         .AT..-...A -..--..... .......C-G ......--CC TA-..T..-- TAT.......
L.R. Canyon              .AT..-...A -..--..... .......C-C .......--C. TG-.....A- CAT.......
Mentone                  .AT..-...A -..--..... .......C-. ......--C. .A-.....-- CAT.......


                          4444444444 444444444
                          2222222223 333333333
                          1234567890 123456789

Blatta orientalis        TGTTTTTGTT AAACAGGCG
Therea petiveriana       ........A. .........
C. kyebangensis          ........A. .........
Cave junction            .......... .........
FSR-4611                 .......... .........
Glendale                 .......... .........
Selma                    .......... .........
Union Creek              .......... .........
Dripping Rock            .......... .........
Mtn. Lake                .......... .........
Powell Gap               .......... .........
Asheville                .......... .........
Highlands                .......... .........
Swain Co.                .......... .........
B.R. Mtn.                .......... .........
Cherry Log               .......... .........
Cheaha St. Park          .......... .........
Lk. Guntersville         .......... .........
L.R. Canyon              .......... .........
Mentone                  .......... .........
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