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Billions and billions sold: Pet-feeder crickets (Orthoptera: Gryllidae), commercial cricket farms, an epizootic densovirus, and government regulations make for a potential disaster

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Abstract

The cricket pet food industry in the United States, where as many as 50 million crickets are shipped a week, is a multi-million dollar business that has been devastated by epizootic *Acheta domesticus* densovirus (AdDNV) outbreaks. Efforts to find an alternative, virus-resistant field cricket species have led to the widespread USA (and European) distribution of a previously unnamed *Gryllus* species despite existing USA federal regulations to prevent such movement. We analyze and describe this previously unnamed *Gryllus* and propose additional measures to minimize its potential risk to native fauna and agriculture. Additionally, and more worrisome, is our incidental finding that the naturally widespread African, European, and Asian "black cricket," *G. bimaculatus*, is also being sold illegally in southern California pet food stores. We assayed crickets of all five USA and European commercial species for presence of the AdDNV to document extent of the infection—all five species can be infected with the virus but only *A. domesticus* is killed. Based on its already cosmopolitan distribution, apparent inability to live away from human habitation, and resistance to AdDNV, we suggest that *Gryllodes sigillatus* is the best-suited replacement cricket for commercial production.

Key words: field crickets, *Gryllus, Acheta*, AdDNV, *Gryllodes*, cryptic species, densovirus, pet food stores, commercial cricket breeders, bar coding, USDA/APHIS

Introduction

The USA commercial cricket industry supplies, yearly, billions of live crickets as food and bait to pet stores, educational facilities, and individuals. Several USA facilities ship 5 million crickets a week (C. Ghann, pers. comm. to DBW, August, 2011) and are important economic engines because they provide many jobs to both on-site workers and the overnight shipping industry. On the web, one can purchase, from several suppliers, 1,000 live crickets for between $10–20. A frequently kept large lizard, such as the bearded dragon, can consume 50–100 adult crickets a day (H. Labe, pers. comm. to DBW, June, 2011). Plus, raising crickets has recently been touted as a way to make easy money (Gillman 2011).

In March, 2011, DBW was asked to review the Center for Plant Health Science and Technology (CPHST) Version 1.0 (Meissner & Ahern 2011) of a United States Department of Agriculture (USDA)-Animal and Plant Health Inspection Service (APHIS)-Plant Protection and Quarantine (PPQ) requested risk assessment for the importation of the field crickets *Acheta domesticus* (L.) and *Gryllus assimilis* (F.) from Europe. This cricket-grower request to import was in response to outbreaks of *A. domesticus* densovirus (AdDNV) in USA commercial *A. domesticus* colonies that had resulted in several cricket operations going into bankruptcy (see Coote 2004 and Hudak 2010, for examples).

This publication is the result of a one plus year investigation involving scientists on six continents, the USDA, agriculture officials in several states, and commercial cricket breeders in both the USA and Europe. It is directed toward the mixed audience of scientists, commercial cricket growers, and government regulators to permit an

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effective, coordinated effort to understand and address problems presented here. While these topics, and target audience, are somewhat disparate, we feel that having all of this information, some of it preliminary, in one place, makes this paper more useful. We report on the five most commercially important worldwide cricket species, describing one of them as new to science based on calling song, morphology, and DNA. All five species have in common the absence of a diapause at any developmental stage, which facilitates their culturing. We also discuss the commercial cricket industry, which cricket species act as hosts for AdDNV (although the virus is only pathological in *A. domesticus)*, argue that federal and state regulatory agencies need better supervision of commercial cricket breeders to minimize potential negative impacts on agriculture and native cricket faunas, and, finally, present some suggestions to improve problems identified by our investigations.

Material and methods

Stridulatory files were examined by removing the right tegmen and placing it under a cover slip. File teeth were counted at 500X magnification with a compound microscope, and an ocular micrometer was used to measure file length across its curve. Tegminal width was measured as maximal distance from medial edge to forewing angle by using the Cu1 vein as a landmark. The tegmen is stored in a gelatin capsule on the same pin as the male.

Calling songs were recorded in the laboratory using a Uher 4000 Report IC reel-to-reel tape recorder with a Sennheiser K34 power module and ME40 microphone. Signals were analyzed on a Tektronix 2214 Digital Storage Oscilloscope and illustrated in Raven Lite 1.0 (Cornell Lab of Ornithology).

DNA extraction, PCR amplification, and sequencing: Genomic DNA was isolated from 95 or 100% ethanol preserved leg tissue using the DNEasy Tissue Kit (Qiagen Inc., 69504). Approximately 500 base pairs of the mitochondrial 16s ribosomal RNA gene were amplified with the primers CGCCTGTTTATCAAAAACAT (forward) and CCGGTTGAACTCAGATCA (reverse). PCR amplification took place in a ThermoFisher PCR Sprint thermocycler in 25 ul reactions using JumpStart REDTaq DNA Polymerase (Sigma, D-8187) with supplied buffer and dNTPs (Sigma, D-7295). Initially, PCR reactions were heated to 94ºC for 5 min, followed by 30 cycles of 94ºC for 1 min, 49ºC for 1 min, and 72ºC for 1.5 min, followed by a further 94ºC for 1 min, 49ºC for 1 min, and 72ºC for 5 min. Negative controls were performed with each reaction. PCR products were visualized on agarose gels, cleaned using the GenElute PCR Clean-up Kit (Sigma, NA1020), and sequenced using the same primers as for PCR on an ABI Prism 377 DNA Sequencer platform with BigDye v.3.1 chemistry at the California State University Northridge DNA sequencing facility.

DNA analysis: Consensus sequences were obtained by manual alignment of forward and reverse sequences using BioEdit v.7.0.5.3 (Hall 1999) and electropherograms viewed using Chromas Lite v.2.01 (http://www.technelysium.com.au/chromas_lite.html). Additional 16s sequences of three species (see Appendix A) were obtained from GenBank. ClustalW (Thompson *et al.* 1994) using default settings and running within BioEdit was used for multiple alignments of all sequences. The final trimmed sequence alignment consisted of either 498 nucleotides (AF248686.1 *G. campestris* (L.) and AF248692.1 *G. ovisopis* Walker from GenBank) or 515 nucleotides (all newly sequenced samples, plus EU557269.1 *Teleogryllus emma* (Ohmachi & Matsuura) from GenBank). DNADIST v.3.5c (Felsenstein 1993) using default settings was used to create a distance matrix. The distance matrix was analyzed using the FastME minimum evolution model (Desper & Gascuel 2002) running on the South of France Bioinformatics Platform web server (http://www.atgc-montpellier.fr/fastme/). As the goal of this analysis was to verify species' identity, and not to provide a robust phylogeny, we opted for a fast distance based analysis of the alignment. The resulting tree (Fig. 6) should therefore be interpreted only as providing support for appropriate species' clustering. A much more comprehensive analysis of North American *Gryllus* phylogeny is forthcoming and will attempt to resolve phylogenetic relationships among all taxa, rather than just the subset of currently named taxa included in the analysis presented here.

AdDNV analysis: Early testing was exclusively on *A. domesticus*. The cricket sources were both from large- and small-scale growers as well as pet stores, most from North America but also some from Europe. Some of the large-scale suppliers were Reeves Cricket Ranch Inc. (WA), Ghann's Cricket Farm (GA), Fluker's Farms (LA), Armstrong's Cricket Farm (LA), Timberline Live Pet Foods, Inc. (IL), Canton Wholesale Bait, LLC (MS), Top Hat Cricket Farm (MI), American Cricket Ranch (CA), and Grigfarm Rotter (Switzerland, now closed). Most crickets that were obtained were dead but we also tested some apparently healthy ones for the presence of AdDNV. On

average, 40 crickets per week were tested over the period from September 2009 to July 2012 starting with AdDNV-containing samples from Washington State. Within a few weeks we received AdDNV-positive samples from across North America.

Parvoviruses, including densoviruses, are exceptionally resistant to proteases and nucleases. A very efficient initial method to purify these viruses is to putrefy the dead crickets for 1–2 days at 4°C after homogenizing in PBS (Tijssen *et al.* 1977). The viral DNA remains protected in the capsid while host nucleic acid and proteins are digested. One volume of chloroform-butanol (1:1) or carbon tetrachloride to 4 volumes of buffer, containing a few crystals of 1-phenyl-2-thiourea, is then added. The supernatant, after centrifugation at 10,000xg for 15 min, contained the virus extracts that can be used for PCR. Samples were retested, only in cases where PCR results were negative, on DNA extracted from 100 or 200 ul of the crude extract using the standard proteinase K-SDS method (Goldenberger *et al.* 1995). The supernatant was diluted 10, 10^2, 10^3, and 10^4 times for semi-quantitative PCR of both the non-structural (NS) and the structural (VP) gene cassettes (PCR fragment sizes of 357 and 304 nts, respectively) according to the method described by Szelei *et al.* (2011). PCR reaction included 2 ul MgCl2 (25 mM), 0.2 mM dNTP, reaction buffer, and Taq polymerase (Epicentre). The PCR program is 95°C for 4 min, 35 cycles of 30 s at 95°C, 30 s at 55°C, and 45 s at 72°C following final elongation 7 min at 72°C.

Note about AdDNV testing. Specimens sent from the USA to Canada (PT) for AdDNV testing by courier service incur heavy custom's clearing costs of over $50 (usually for a few dead crickets). So far PT has spent over $25,000 for clearing of crickets. Please note that PT has subsequently transferred the exact testing method to Mississippi State University (for contact: Dr. Amanda Lawrence [ALawrence@entomology.msstate.edu] to avoid these USA to Canada custom's charges.

Results

The Crickets

Acheta domesticus
(Fig. 1)

Prior to 1977 cricket breeders in both the USA and Europe almost exclusively raised the (European) house cricket, *A. domesticus*, already a cosmopolitan species (Weissman & Rentz 1977; Weissman *et al.* 1980; Walker 2012). Starting in Europe in 1977 (Szelei *et al.* 2011) and North America in 1988 (Styer & Hamm 1991), production facilities were infected by AdDNV, although severe epizootics were not apparent in North America until 2009/2010 (Liu *et al.* 2011). Certain USA breeders, such as Armstrong's Cricket Farm (http://www.armstrongcrickets.com/), through a strict program of "reverse" isolation, continue to successively raise *A. domesticus* because their facilities have not been infected (see Table 2 below and Discussion), but they are the exception (C. Ghann, pers. comm. to DBW, September, 2011).

Although *A. domesticus* has been feral in the USA for years (Weissman & Rentz 1977), recent local adaptation has been observed (DBW, unpublished) in many populations on two fronts: 1) twenty years ago, adult male field calling songs were quiet and intermittent and were easily identified without having to capture the singing male. In the last five years, chirp in *A. domesticus* have become more regular and louder and have resulted in increased collecting efforts to confirm identification since singing males can sound like a *Gryllus* species, and 2) populations have impacted native *Gryllus* species, for example at Furnace Creek, Death Valley National Park, California (DBW, unpublished) where in the early 1980s, *A. domesticus* was rare and a native *Gryllus* was common. Repeat collecting at Furnace Creek over the years, most recently in 2003, yielded hundreds of *Acheta* and only two native *Gryllus* individuals. Elsewhere, *A. domesticus* can be found away from human habitation in natural USA habitats (Weissman & Rentz 1977).

Gryllodes sigillatus (Walker)
(Fig. 1)

Variously called the tropical or Indian house cricket, *Gryllodes sigillatus* is also a cosmopolitan species, and we

have found it sold in both USA and European pet food stores. This species is easily distinguished from *A. domesticus* by its song, shorter tegmina and hind wings, longer cerci, and greater agility and ability to jump. In contrast to *A. domesticus*, populations are almost unknown from natural habitats (DBW & DAG, unpublished) and are always associated with human structures. This cricket species is easier to culture than *A. domesticus* (Scott Sakaluk, pers. comm. to DBW, January, 2012).

FIGURE 1. *Gryllodes sigillatus* (adult male left) and *Acheta domesticus* (adult male right). Both species are usually light brown/tan in color with a black bar between the eyes. Adults of both sexes of *G. sigillatus* always have short tegmina (top wings) covering about half of the abdomen. Adults of both sexes of *A. domesticus* have longer tegmina reaching near tip of abdomen and hind (bottom) wings (see arrows) that extend beyond tip of abdomen. In culture, some adults of the latter species shed their hind wings (Walker 1977). Such an occurrence can be confirmed by the absence of any hind wing or the presence of just a stump.

Gryllus assimilis
(Fig. 2a, b)

Originally described from Jamaica in 1775 where it is one of three native *Gryllus* species on the island (Weissman *et al.* 2012), this taxon goes by various common names: Jamaican field cricket, Jamaican brown cricket, and brown silent cricket by European breeders (e.g. Bugs-International of Germany). *G. assimilis* is also presently known from several Caribbean Islands (Otte & Perez-Gelabert 2009), southern Texas, the east coast of Mexico south into Costa Rica, and possibly into South America (Weissman *et al.* 2009). The species is introduced in south Florida (Alexander & Walker 1962). Many commercial breeders in Europe claim to sell *G. assimilis*, but they are actually selling *G. locorojo* **n. sp.** (see below). In fact, we are unable to document any European dealer selling verified *G. assimilis*. In contrast, several USA cricket farms have USDA approved, verified (by DBW) *G. assimilis* cultures. These growers were originally supplied (pers. comm. to DBW by several growers, spring, 2012) "starter crickets" by Anthony Zera (University of Nebraska), who began his cultures (pers. comm. to DBW, November, 2011) with specimens supplied by Thomas Walker (University of Florida) in 1992 and collected in Gainesville, Florida (see Alexander & Walker 1962). A. Zera reports (pers. comm. to DBW, November, 2011) that he gets some six generations per year of *G. assimilis* when raised at 28–30°C. After some 120 generations, he notes no signs of inbreeding depression or changes in calling song. Its distinctive calling song consists of 6–10 pulses/chirp given at 1–2 chirps/second all with a pulse rate greater than 70 at 25°C. *G. assimilis* has a morphologically indistinguishable sister species, *G. multipulsator* Weissman, the latter known from southern California, southern Nevada, Arizona, Baja California, Mexico, and along the Mexican mainland west coast (Weissman *et al.* 2009).

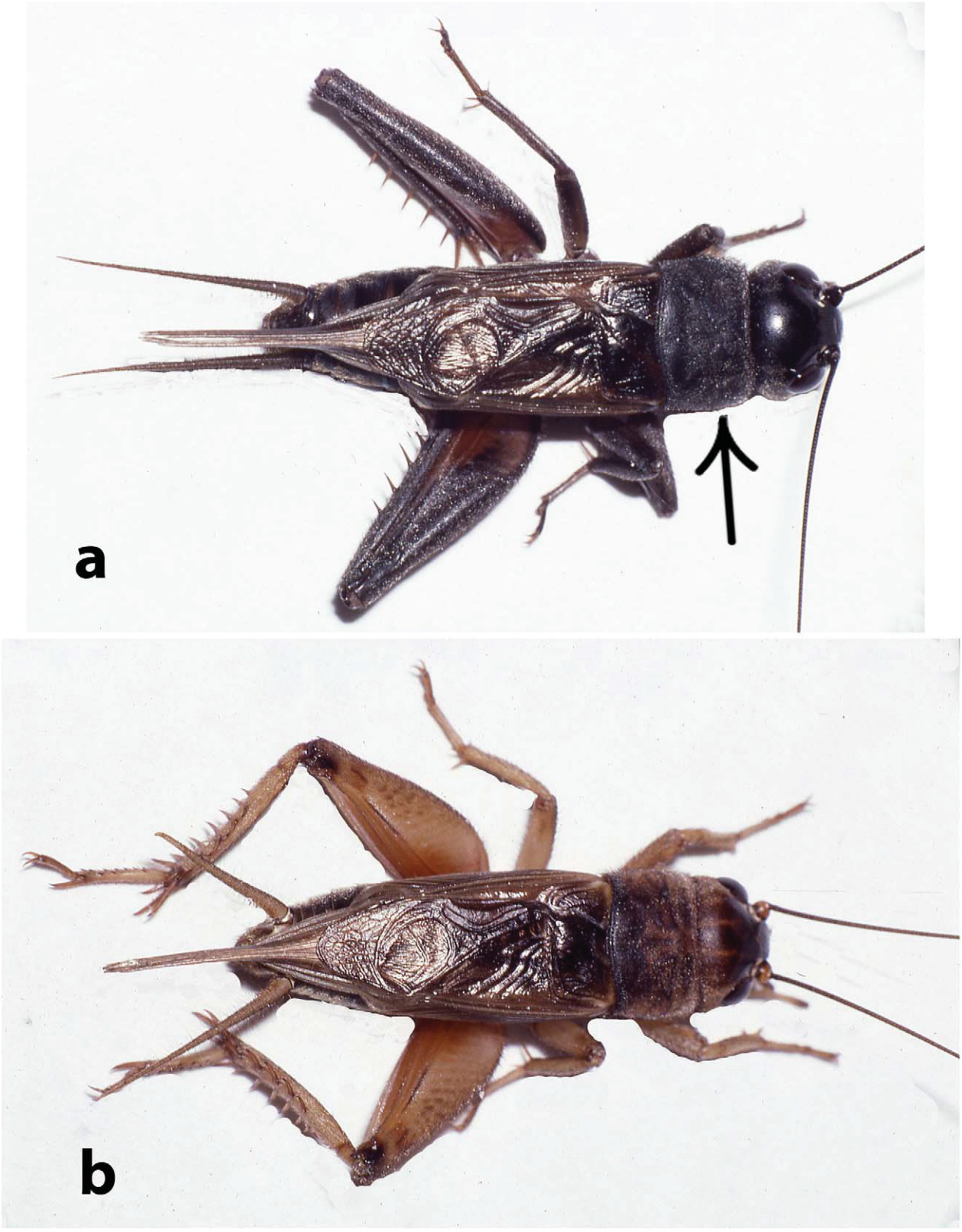

FIGURE 2. *Gryllus assimilis*. a. Adult male from Mexico, Quintana Roo, near Cobá, more typical dark phase. Note dull pronotum (arrow) due to covering of fine hairs. b. Adult male from Mexico, Quintana Roo, Cancun, rarer light color phase, showing dull pronotum and head with indications of stripes. Field collected adults of both sexes are always long winged. Such color variation in natural specimens confirms the importance of male calling song for positive identification.

***Gryllus bimaculatus* De Geer**
(Fig. 3a, b)

Commonly called the two spotted cricket or, by European breeders, the black cricket, *G. bimaculatus* apparently is the most widely distributed *Gryllus* species and is found from the tip of South Africa north into Europe and east as far as Thailand (Otte & Cade 1984). This is a medium-large sized, short hind femur, usually pure black, short or long hind winged cricket with a shiny pronotum. Most males have a pale area (Fig. 3a) at the base of each tegmen where they attach to the pronotum. Adult females may be without or have a slight indication of pale tegminal areas (Fig. 3a). Brown males are known (see Fig. 3b, and Otte and Cade 1984). Song with 2–6 pulses/chirp, usually 3–5 chirps/second, pulse rate 21–28 at 25°C. Table 1 presents morphological and song parameters measurements from examined localities.

G. bimaculatus is readily available in European pet food stores, and we recently discovered them for sale in San Diego, California (DBW personal observations). Because of an apparently broad ecological tolerance as indicated by its widespread distribution, we feel this taxon is more likely than *G. locorojo* **n. sp.**, to become an established agricultural pest in the USA. Supporting our concern, Smit (1964:79) notes that *G. bimaculatus* "….feeds on many kinds of vegetables" and can "eat the bark off young fruit trees." As Meissner & Ahern (2011) state: "This species [*G. bimaculatus*] is a quarantine pest in the United States and its introduction into the United States is unacceptable." Yet the USDA has known that this San Diego, California, pet food store has been selling this cricket since late May/ early June, 2012, but has taken no action to date (as of mid-August, 2012). Since the larger, commercial supplier of this cricket is also unknown, it is likely that many more USA pet food stores are selling *G. bimaculatus* since most commercial dealers sell to many retail stores.

TABLE 1. Body measurements (mm) and song parameters of *Gryllus* species discussed in this paper.

	MALES				
SPECIES	teeth in file	file length	teeth/mm	tegmina length	tegmina width
G. locorojo (n=40)	150–189	3.0–4.0	44.7–52.9	13.4–16.4	3.9–5.0
G. bimaculatus (n=12)	115–162	3.5–5.2	31.0–34.7	14.7–19.1	5.0–6.5
G. bimaculatus	140–160				
G. argentinus	159–205	4.16–5.1	36.5–45.6	13.6–16.6	5.05–6.35

continued.

			SONG		
SPECIES	hind femur length	cercus length	pulses/chirp	chirps/second	pulse rate
G. locorojo (n=40)	10.77–12.92	9.25–10.82	1–3	0.5–1.5	25.0–41.7
G. bimaculatus (n=12)	9.41–11.18		(2) 3–5 (6)	2.5–4.5	20.8–27.8
G. bimaculatus			3–5	3	26–30
G. argentinus	11.1–13.05		2 (3)	1–2.5	17.5–22.7

continued.

	FEMALES			
SPECIES	hind femur length	cercus length	ovipositor length	SOURCE
G. locorojo (n=40)	9.32–13.74	8.96–11.37	10.34–13.84	this report
G. bimaculatus (n=12)	9.49–10.82		11.32–12.67	this report
G. bimaculatus				Otte & Cade (1984)
G. argentinus				Pinho Martins & Zefa (2011)

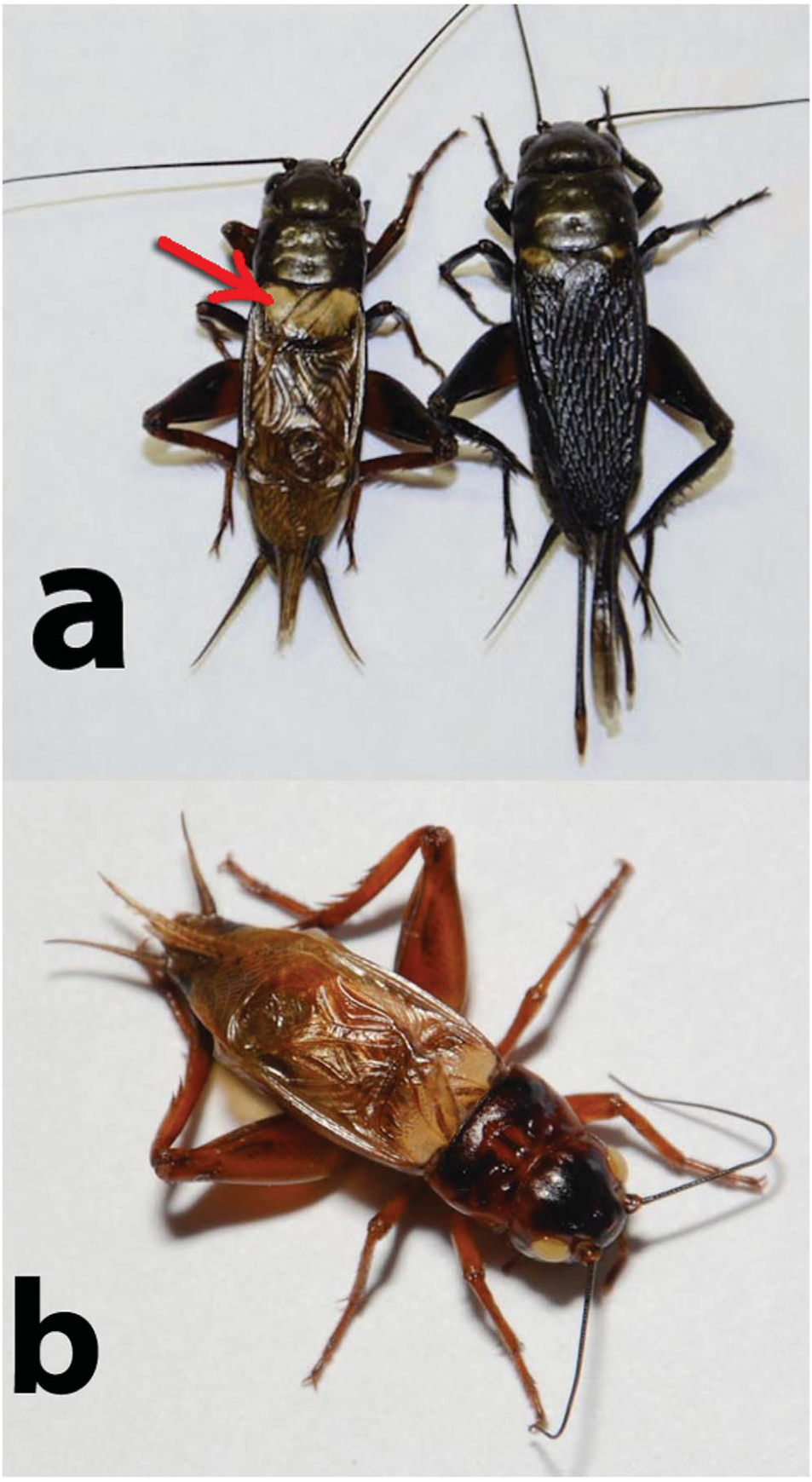

FIGURE 3. *Gryllus bimaculatus*. a. Adult male (left) and adult female (right) both from pet store, San Diego Co., California. Note shiny pronotums and prominence of "bimacula" areas (red arrow pointing to left macula) at base of tegmina, although typically less so in the female. b. Adult male, more unusual light colored phase with cream colored eyes, from pet store in France. DNA profiles of both these "populations" are shown in Fig. 6.

***Gryllus locorojo* Weissman and Gray n. sp.**
(Figs. 4a, b, c, d, 5a, b, Table 1).

Recognition characters. Known only from pet food stores and commercial cricket growers in the USA, Europe, and Russia. Body length medium-large, long or short winged, typically reddish/brownish colored head (Fig. 4a) with three or four longitudinal stripes visible even in specimens with darker heads. Cerci short (see Table 1). Pronotum dull or shiny. Song (Fig. 5a, b) variable, usually two pulses/chirp (range 1–3), usually less than one chirp/sec but some males sing at 2–3 chirps/sec. Pulse rate 25–42 at 25°C. Song different from any known USA, Mexican, or Central American *Gryllus* spp. (Weissman & Gray, in prep.). Most similar *Gryllus* song is *G. argentinus* Saussure (Martins & Zefa 2011) from Brazil and Argentina but chirp rate higher and pulse rate lower in latter taxon (see Table 1 for comparison; also Shestakov & Vedenina [2012]). Also differs in color pattern (*G. argentinus* has a solid black head and pronotum) and DNA (see Fig. 6). In USA and Mexico, most similar cricket song is from the non-native *Acheta domesticus*.

Holotype. Male: USA: California, Los Angeles Co., Compton, Rainbow Mealworms, 126 E Spruce St., 90220. December, 2011. DBW S(top)11–124. R(ecording) 12–1,5. DNA sample G2219; 16s ribosomal RNA gene GenBank accession #JX269046. Type deposited in California Academy of Sciences (CAS), Entomology Type # 18657.

Paratypes. (Total: 50♂ 22♀). Bassett's Cricket Ranch, DBW S11–109, Visalia, California, x-2011, 6♂; Ghann's Cricket Farm, S11–117, Augusta, Georgia, xi-2011, 9♂, 4♀; American Cricket Ranch, S11–122, Lakeside, California, ii-2011, 6♂, 8♀; Rainbow Mealworms, S11-124, Compton, California, xii-2011, 3♂ 6♀; Tobias Valentin, S12-2, Copenhagen, Denmark, xii-2011, 5♂, 4♀; Monkfield Nutrition Ltd., S12-15, Herts, England, v-2012, 21♂. All paratypes deposited in CAS.

Description. See Table 1 for measurements. Genitalia (Fig. 4b, c) typical of *Gryllus* with male epiphallus three lobed with a longer, slender median lobe. Tegmen with 4–5 harp veins (Fig. 4d).

Etymology. Given the common name moniker "crazy red" in early discussions by Clay Ghann, herein formalized as its scientific name.

Remarks. Biology unknown. No apparent diapause when raised under commercial conditions of 28°C, 40% relative humidity, and an 11 hour light/13 hour dark cycle. In Europe this previously unrecognized cricket has long been known as "*G. assimilis*" or the "brown silent cricket." In Denmark, it is called the "Steppe cricket" (T. Valentin pers. comm. to DBW, January, 2012). In Russia, it is called the "banana cricket" (Shestakov & Vedenina 2012). Its DNA (Fig. 6) and song are very different from true *G. assimilis* (see above).

According to Varvara Vedenina, Russian Academy of Sciences, Moscow (pers. comm. to DBW, January, 2012): "The cricket culture under the name "*Gryllus assimilis*" came to the Moscow Zoo from the Berlin Zoo in the beginning of the 1990s. No details are known. A bit later, in 1997, the cricket eggs under the name "*Gryllus argentinus*" came from Paris Museum of Natural History to St. Petersburg. These eggs definitely originated from Ecuador, since French colleagues returned from an expedition there. Both cultures appear to be identical." Unfortunately, Dr. Vedenina was unable to get more information from the Paris Museum of Natural History about the origin of their eggs.

DNA analysis

Figure 6 shows the results of the FastME distance DNA analysis. *G. locorojo* samples (see Appendix A for locality data) from throughout the pet-trade cluster together, as do the *G. bimaculatus* samples. The most striking result from our analysis of the *G. locorojo* 16s sequences is the 100% genetic uniformity of all samples across the 515 base-pairs; all 28 sequenced individuals were identical at all nucleotide positions. They are also different from any known North American *Gryllus* (including more than 80 undescribed taxa from USA, Mexico, and Central America [Weissman & Gray, in prep.]). Additionally, these specimens map (Fig. 6) into the New World clade along with 15 other previously described New World *Gryllus* taxa. While these data do not reveal a source area for *G. locorojo*, they suggest (and see comments above under *G. locorojo*) the following narrative: *G. locorojo* was imported into Europe in the late 1970's or 1990's, probably from South America (Ecuador?), when European *A. domesticus* was infected by AdDNV. We believe they were subsequently exported to the USA in 2009 when AdDNV impacted USA commercial breeders.

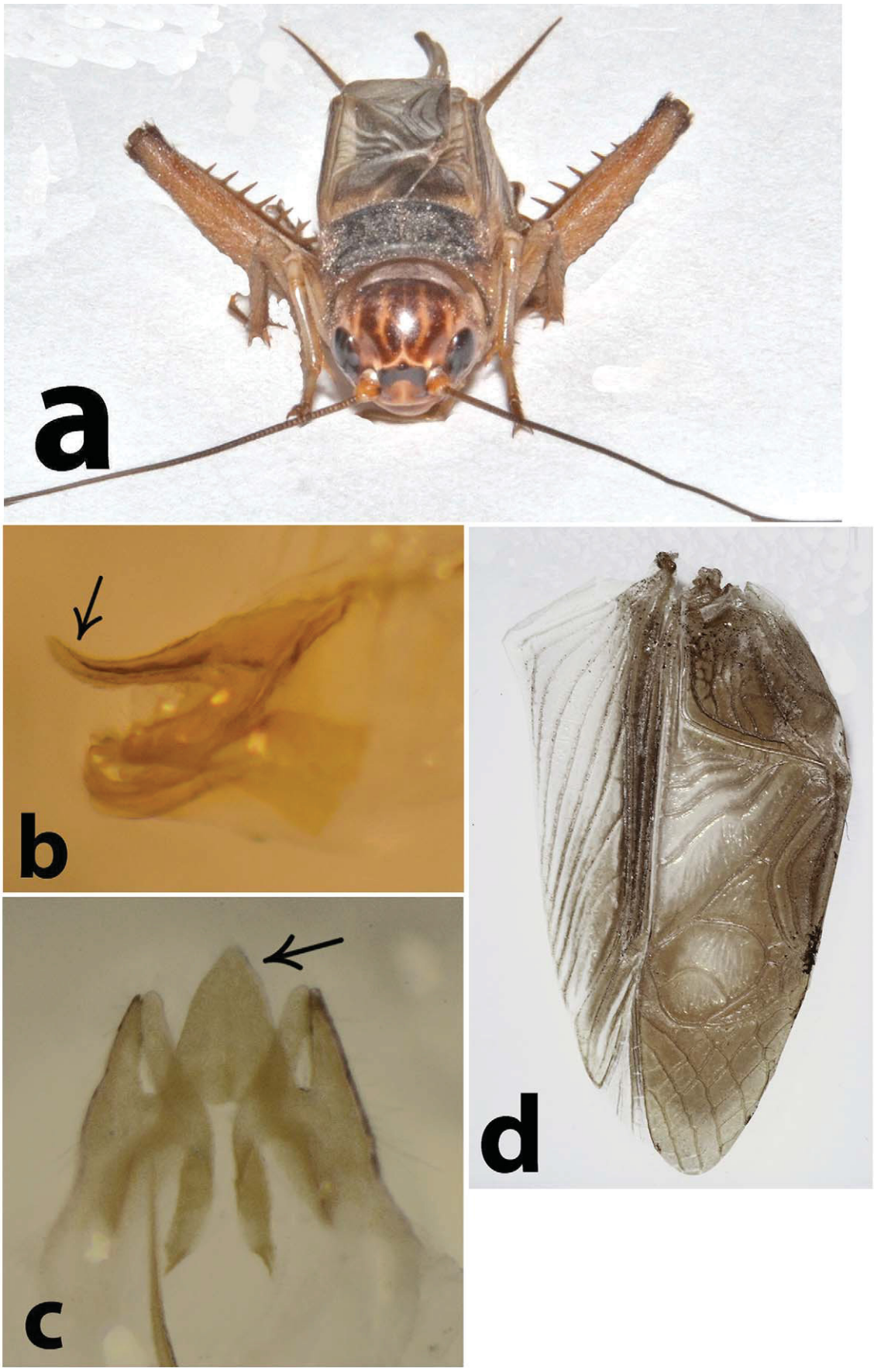

FIGURE 4. *Gryllus locorojo* a. Adult male from Ghann's Cricket Farm showing characteristic head stripes. Adult male genitalia consistent with *Gryllus:* b. lateral view (arrow points to middle lobe) and c. ventral view (arrow points to middle lobe). d. Right adult male tegmen.

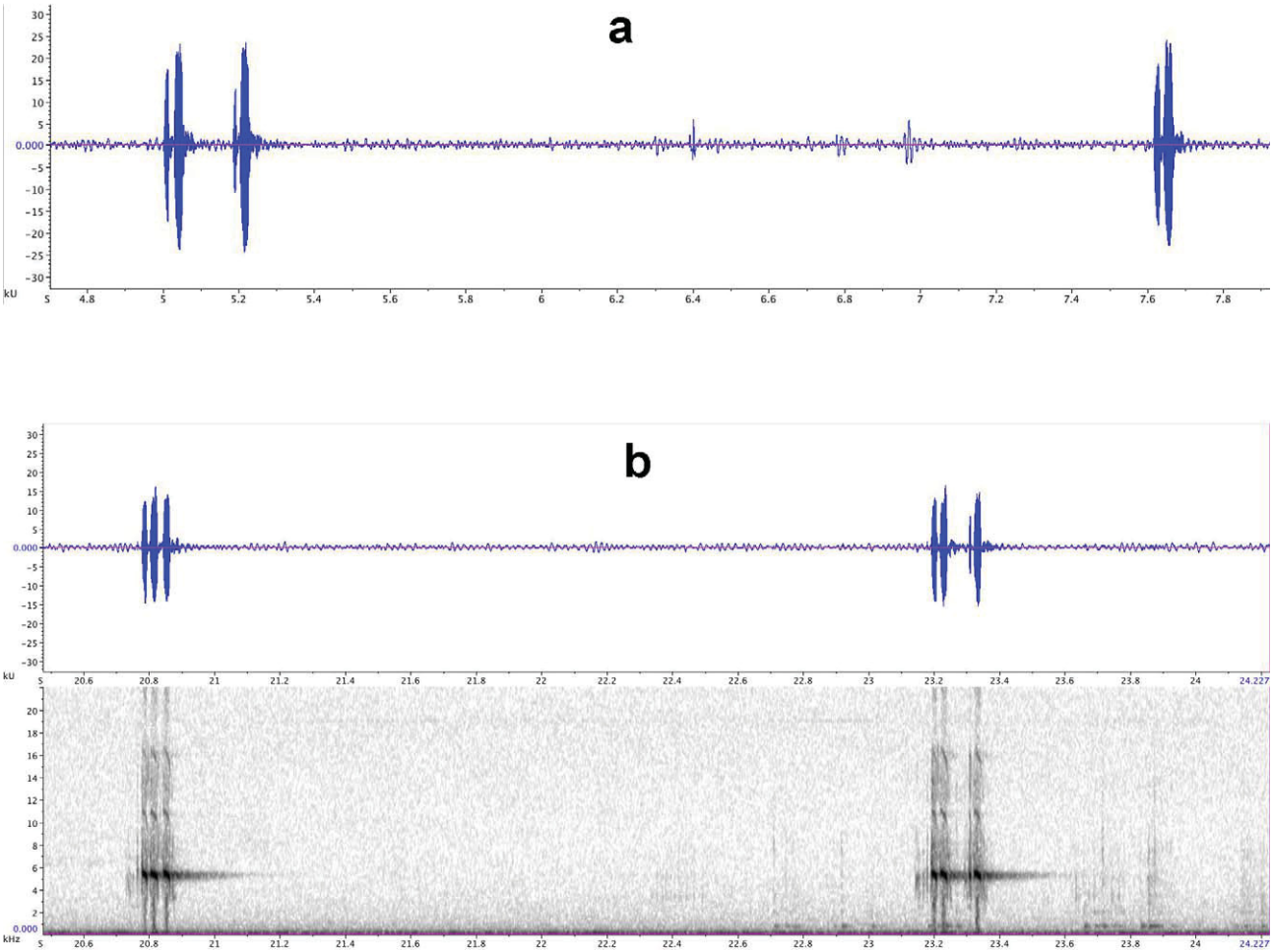

FIGURE 5. Oscillograms (a,b) and sonogram (b) of the calling song of *G. locorojo.* a (upper). Male from S(top) 11–124, Rainbow Meal Worms, R(ecording) 12–4 @ 23°C showing only pairs which are sometimes grouped. B (lower). Male from S12–15, Monkfield Nutrition, R12–19 at 24°C, showing a triplet, doublet, and singlet pulse chirps with a dominant frequency of 5.4 kHz (Interestingly, dominant frequency is 4.2 kHz in Shestakov & Vedenina 2012).

This finding of genetic uniformity is also significant because it indicates the lack of apparent hybridization since many breeders raise two species of crickets together in the same bins (pers. comm. to DBW by several commercial cricket growers in 2011 and 2012). In contrast, *G. bimaculatus* samples were not identical and show some suggestion of geographic separation among samples from southern Africa and Europe (Fig. 6). Although our simple phylogenetic analysis was not intended to resolve the bigger picture of *Gryllus* phylogeny, it appears from this analysis that *G. locorojo* has closer affinity to New World *Gryllus* than to the Old World *G. bimaculatus* and *G. campestris* Linnaeus.

The cricket farms

The exact number of large cricket "factories" in the USA is unknown, but probably somewhere around 30 (C. Ghann, pers. comm. to DBW, January, 2012). There are also an unknown number of much smaller operations. Additionally there is an informal breeders' group that has monthly conference calls. Called the CHIRP group (for **C**ricket & **H**erptile **I**ndustry **R**ecovery **P**artners group), they discuss problems within the industry and their facilities. What is usually not discussed between growers is the source of their crickets. Some operations merely distribute crickets that are raised by others. Most breeders are located in the southeastern USA (C. Ghann, pers. comm. to DBW, January, 2012), but a web search finds five commercial breeders in California. Most, if not all

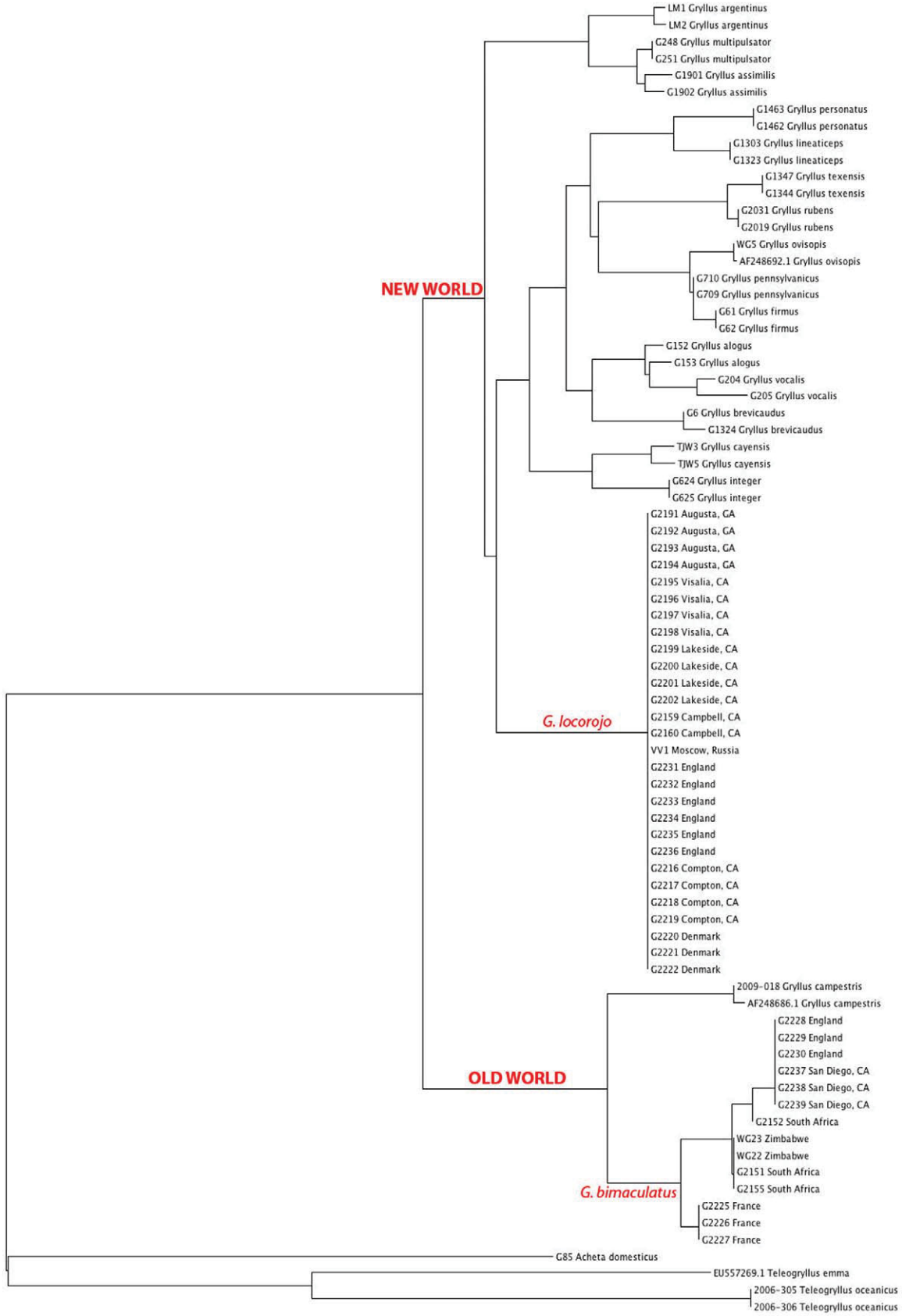

FIGURE 6. Fast distance based analysis tree for 16s ribosomal RNA gene. Note total genetic uniformity among 28 individuals of *G. locorojo* from eight "localities" on three continents. See Appendix A for specimen source data.

USA breeders apparently supply hundreds of pet stores and individuals each week. As an example, Bassett Cricket Ranch in Visalia, CA, notes on their web site that they "produce in excess of 3 million crickets a week, primarily for the pet industry" (http://www.bcrcricket.com/about.html). At any one time, they have 40 to 60 million live crickets of all sizes on site (R. Bassett, pers. comm. to DBW, December, 2011). With a life cycle of two weeks from oviposition to egg hatch (crickets are raised between 28–32° C) plus another six weeks until molting to adult, Bassett can have five to six generations per year and was able to increase some 1,500 "starter" *G. locorojo* crickets into 40 million in 18 months. And they supported this above activity while concurrently selling several million crickets a week.

The physical size of these commercial operations ranges from 100 to 10,000 m^2 (Szelei *et al.* 2011).

The virus

Densoviruses are small, nonenveloped viruses that contain a linear, single-stranded DNA (Szelei *et al.* 2011). They are usually highly pathogenic in their natural hosts (Fédière 2000). Transmission of AdDNV is normally by the fecal-oral route but collected aerosols also suggest this route is possible (Szelei *et al.* 2011). The virus can survive on the cuticle of a cricket for months. Once a cricket breeding facility is infected, subsequent virus elimination appears impossible, short of nuclear destruction (pers. comm. to DBW, February, 2012, from several cricket growers). Soil isolates taken 30 m from breeding facilities are positive for the virus (Tijssen, unpublished), and virus is readily isolated from filters in affected farms (Szelei *et al.* 2011). AdDNV has its highest mortality in last instar and young adult *A. domesticus*, and these specimens have almost completely empty guts (Liu *et al.* 2011). The complete molecular characterization of this virus showed that it has unique features among parvoviruses such that it is classified in its own genus (Tijssen *et al.* 2011). X-ray crystallographic data have recently been obtained to describe the 3D near-atomic structure of this virus (M.G. Rossmann & P. Tijssen, unpublished).

Virus sampling

TABLE 2. Results of *Acheta domesticus* densovirus survey.

Cricket Species	Source Location	No. tested	% positive among tested	AdDNV susceptibility
Acheta domesticus (Ad)	98% from growers	3200	98#	4+ positive
Gryllodes sigillatus (Gs)	30% from growers	40	95	4+ positive
*Gryllus assimilis** (Ga)	98% from growers	300	10	1+,3+ positive
Gryllus bimaculatus (Gb)	from pet stores	70	5	1+ positive
Gryllus locorojo (Gl)	85% from growers	500	92	4+ positive

1+ POSITIVE: after a 10-fold dilution, positive but not at higher dilutions.
3+ POSITIVE: after a 1,000 times dilution, sample still positive but not at higher dilutions.
4+ POSITIVE: positive after at least 10,000 times dilution.
Source locations: usually large USA growers as indicated in Materials and Methods.
Samples of *A. domesticus* from Armstrong's Cricket Farm tested continuously negative.
Pet stores, also those supplied by reputed growers such as Bugs-International (Germany), often sold positive specimens.
* Only once, *Gryllus assimilis* tested 3+ positive; other tests were either negative or, for 10% of the specimens, 1+positive .
Samples included some specimens from France (Ga, Ad), Denmark (Gl) and England (Gl, Gb, Gs)

Table 2 summarizes the results of AdDNV sampling and shows that at least four of the five commercial cricket species are suitable hosts for the virus, although *G. assimilis* was usually infected to a much lower degree and strong contamination could not be excluded. In fact, small-scale infection assays of *G. assimilis* from the laboratory of A. Zera were negative (Szelei *et al.* 2011). Similarly, only 5% of *G. bimaculatus* (from two locations: one in France and one in San Diego, CA) tested positive at the lowest dilution and appear, so far, resistant. The interpretation of PCR results (Fig. 7) required distinguishing between levels of ingested virus particles, e.g. fecal-

oral route or cannibalism, and replicating virus. Previously, we observed that ancillary insects in cricket operations, where a high percentage of *A. domesticus* were infected, other insects, including "waxworms" (*Galleria mellonella*), mealworms (*Tenebrio molitor*), and "superworms" (*Zophobas morio*) tested sometimes positive at a 10-fold dilution despite being resistant (Szelei *et al.* 2011). From Fig. 7 it is also clear that some samples contain inhibitors when bands are stronger at higher dilutions (cf. lane 11 and 12 of Fig. 7).

The possibility of diluting the samples 10,000x or more for the other species and still obtaining strong PCR bands in a high percentage of specimens indicates a high level of virus replication in these individuals.

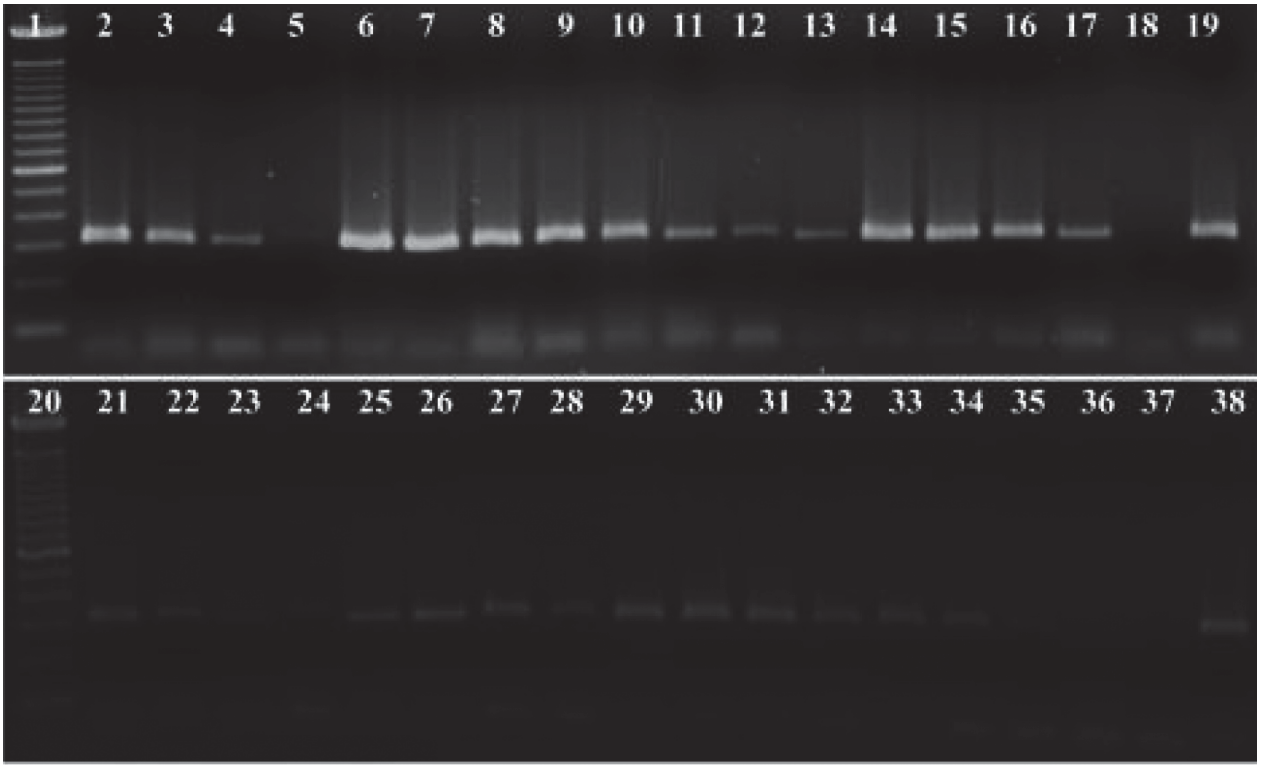

FIGURE 7. Detection of AdDNV by PCR analysis for VP [capsid protein] primers (results identical for NS [nonstructural] primers and thus not presented). Lane 1 and 20: 100 bp ladder (InVitrogen Inc.).
Lane 2–5: *Gryllus assimilis*; dilution: 10, 10^2, 10^3, 10^4, respectively
Lane 6–9: *Acheta domesticus*; dilution: 10, 10^2, 10^3, 10^4, respectively
Lane 10–13: *Gryllus locorojo*; dilution: 10, 10^2, 10^3, 10^4, respectively
Lane 14–17: *Acheta domesticus*; dilution: 10, 10^2, 10^3, 10^4, respectively
Lane 18: negative control
Lane 19: positive control
Lane 21–24: *Acheta domesticus*; dilution: 10, 10^2, 10^3, 10^4, respectively
Lane 25–28: *Gryllus locorojo*; dilution: 10, 10^2, 10^3, 10^4, respectively
Lane 29–32: *Gryllodes sigillatus*; dilution: 10, 10^2, 10^3, 10^4, respectively
Lane 33–36: *Gryllodes sigillatus*; dilution: 10, 10^2, 10^3, 10^4, respectively
Lane 37: negative control
Lane 38: positive control

The government regulators

All versions of the USDA risk assessment report associated with the importation and distribution of these crickets noted several cogent points (Meissner & Ahern 2011, 2012):

1. Whatever commercial species of cricket is approved to import, and wherever from, expect them to be introduced into the environment through accidental escape or intentional release. [This probability is especially

likely since most cricket farms are located in mild weather, southern USA locations, and most New World *Gryllus* species seem to be pre-adapted to warm climates given their natural distributions and absence of any diapausing stage (DBW, unpublished data)].

2. The potential effects of such releases on the environment, agriculture, and native crickets are unknown, including introducing or spreading pathogens or parasites.
3. The identity of any European-imported or USA-moved cricket should be confirmed by an appropriate expert. [The authors of this paper feel that, at the minimum, this identification requires morphological and calling song analysis by someone that is actively working on *Gryllus*. Unfortunately, simple one-gene "bar code" comparative DNA analysis (typically using the COI locus) will be inadequate since our work with North American *Gryllus* demonstrates that even two genes (one of which is COI) are not adequate for separating many *Gryllus* taxa (Weissman & Gray in prep.)].

On September 7, 2011, a conference call was held to discuss this situation. Participating were academics (DBW, DAG, & T. J. Walker of University of Florida) and USDA personnel from the states of Washington, Pennsylvania, and Maryland plus Oregon Department of Agriculture personnel. The participants were told that USDA had, so far, issued only three permits nationwide for interstate transport of "*G. assimilis,*" and none for any breeder in California. Unfortunately, at the time of that conference call, and apparently unknown to USDA, at least three California cricket breeders (Bassett Cricket Ranch, Rainbow Mealworms, and American Cricket Ranch) were already shipping these crickets to hundreds of locations both within California and to other states. In all three cases, their crickets had been previously misidentified by USDA as *G. assimilis* when, in fact, we determined they were the very different, and unrecognized *G. locorojo*.

Two specific recommendations arose from this conference call, which were largely incorporated into the subsequent USDA document, dated June, 2012, (Meissner & Ahern 2012):

1. All *Gryllus* species should be phased out from all USA commercial operations.
2. Either *Gryllodes sigillatus* or USA virus resistant *Acheta domesticus* should be substituted for those *Gryllus* crickets now in culture. Because of the information given above under *A. domesticus* and *G. sigillatus*, the conference-call scientists favored the latter over the former as the cricket farm species of choice.

To complicate matters, regulatory conflicts exist between USA and state agencies. For instance, Section 3558 (http://ucanr.org/sites/plantpest/files/63513.pdf) of the California Department of Food and Agriculture Plant Quarantine Manual states:

> "Section 6305 of the [U. S.] Food and Agriculture Code requires persons to obtain a permit from the director or the United States Department of Agriculture to import into, or ship or transport within, the state live insects except for certain exemptions. One of these exemptions is for beneficial or useful insects of common occurrence in the state. To identify which beneficial insects do not require a permit to import into, or to ship or transport within, the state the following lists are provided."

These lists, which run for more than two pages, include the following listing: "common black field cricket – *Gryllus* sp." Because there is no California or USA species of *Gryllus* to which this common name is currently applied, growers could argue that any *Gryllus* species that has any black individuals at any time during its lifetime and that inhabits the USA, would be covered under this carte blanche decree and allowed into California, or shipped within California, without permits. These lists also include 23 orders of arthropods, of which 11 are insects. They are all given unobstructed importation credentials without the need for regulatory review of any kind.

Surprisingly, no one representing California, whose state agricultural interests probably have more to lose than any other state should *G. locorojo* or *G. bimaculatus* escape and become a feral pest, has been involved in any of these deliberations. And continued worldwide climate change could put more northern California agricultural areas at future risk from feral crickets.

Discussion

Prevalence of densovirus and implications for industry. Testing of AdDNV-containing samples started in September, 2009, with diseased *A. domesticus* crickets from Washington State. Within a few weeks AdDNV-positive *A. domesticus* samples from across North America, from facilities in California, Georgia, Ontario and Quebec, were obtained. In a previous study on sequences of all AdDNV isolates from the last 40 years, Szelei *et al.* 2011 estimated that European and North-American AdDNV strains diverged after 2006. This North American epizootic may thus have been building up for about three years, since Liu *et al.* (2011) report the first significant outbreaks in 2009/2010.

Over the last two years, on average, about 40 *A. domesticus* specimens per week were received, virtually all from cricket farms. More than 95% of the samples were very strongly positive with the simple, crude extraction method. An additional 3% were positive when the viral DNA was purified. The PCR sensitivity of the extraction method was about 1,500 genome copies as established by spiking a negative sample with known amounts of viral DNA. DNA purification from the extract improved the sensitivity to about 30 genome copies mainly by removing PCR inhibitors. The fraction of the extraction buffer used (1/1,000), the dilution factor (10,10^2,10^3, and 10^4), and the PCR sensitivity gave a rough estimate of the number of genome copies per cricket (if 100% of virus was extracted). At least 1.5 billion virus copies were present per cricket if the endpoint was a dilution of 10^3. Positive samples were, with rare exceptions, strongly positive, i.e. more than 15 billion (perhaps even one trillion) virus particles per cricket. One mg of virus equals 100 trillion AdDNV particles. The lower limit, i.e. positive at a dilution of 10, represents 15 million virus particles per cricket or 300,000 particles if the DNA was purified. Probably 1,000 particles are sufficient to infect a cricket (Tijssen, unpublished data).

During the last nine months, species other than *A. domesticus* were submitted for testing for AdDNV, probably as a result of high mortality rates among house crickets and desperate growers looking for alternatives. All species were found to be susceptible to AdDNV (Table 2), albeit to a variable degree. *Gryllus locorojo* and *Gryllodes sigillatus* that were received were infected to a similar degree as *A. domesticus* (similar virus load).

Monocultures and high-density cultures of multiple species of crickets pose special problems. A cricket species that is hardly susceptible may become a preferred target after one or more mutations in the viral capsid. A prime example of a new parvovirus, which is related to densoviruses, and epizootic with high morbidity and mortality, was the sudden appearance of canine parvovirus causing a pandemic among dogs in the 1970s after very few mutations of the cat parvovirus (Parrish & Kawaoka 2005). Thus the search for an alternative cricket species may thus be a short-term solution if AdDNV should continue to mutate. On the other hand, crickets with decreased susceptibility to AdDNV may avert further disasters in the industry since infected, but otherwise lively, crickets definitely do not pose a danger to any vertebrate pets. Significantly, to date, no breeder has reported AdDNV mortality problems in any cricket species other than *A. domesticus*, even in those cases where cricket species show significant levels of infection.

Regulatory issues and suggestions. Why has USDA lost complete control and created a potential regulatory nightmare?

1. From various discussions with cricket farm owners, many, but certainly not all, have little or no interest in what species they are rearing. Nonscientists may also be unaware that common names (vs. scientific names) frequently are erroneously applied to different, formally named species.
2. Ignorance on the grower's parts and lack of policing of the growers by USDA as to the regulations and risks of unauthorized cricket movement.
3. The USDA currently has no expert in cricket taxonomy on staff. This lack of expertise is coupled with a hesitancy and/or lack of funds to consult outside experts as validated by early misidentification of *G. locorojo* as *G. assimilis* since there are several outside USA experts who would not have made such a error.

To remedy this situation, we propose the following:

1. As quickly as possible, elimination of all cultures of *G. assimilis* and *G. locorojo*, regardless of origin, in order to minimize the possibility of either species becoming established in the USA (see similar reasoning in Simberloff 2012).

2. The substitution of these commercial populations of *Gryllus* spp. with, preferably, *Gryllodes sigillatus* or, alternatively, USA origin, virus-resistant/tolerant *A. domesticus*.
3. The immediate elimination of all USA cultures of *G. bimaculatus*.

To achieve these goals, we suggest that USDA/APHIS and CHIRP should agree to a jointly funded, one to two year transition period to allow time for "commercially adequate numbers" of *Gryllodes sigillatus* or *A. domesticus* to be achieved by all growers. During the period to build up such cultures, these two cricket species will be kept physically separate from cultures of all *Gryllus* species, which does not now routinely occur. We propose a joint fund because early USDA misidentifications resulted in some growers spending significant funds establishing cultures of non-approved cricket species. Once adequate numbers of *Gryllodes* and/or *Acheta* are obtained, all residual cultures of *Gryllus* should be destroyed.

Future efforts should be directed by USDA/APHIS at educating growers of policies and reasons for such statutes, eliminating conflicts between Federal and State laws, and designating qualified people to certify that growers are using permitted species.

Kudos should be given to those growers (see Fig. 8) who have incurred extra expenses while voluntarily staying within the law and to those growers (see Fig. 9) who have been able to continue growing "*Acheta domestica*" (sic).

Acknowledgments

We thank the many colleagues, commercial breeders, and pet store owners who have contributed to this international effort.

In particular, for sending material for DNA analysis, we thank Varvara Vedenina (*G. locorojo* -Moscow), Tobias Valentin (*G. locorojo* -Denmark), Luciano de Pinho Martins (*G. argentinus*), Thomas J. Walker (*G. firmus,* and *G. cayensis*), Sean E. Walker (*Teleogryllus oceanicus*), and Tom Tregenza (*G. campestris*). The following commercial cricket breeders also sent material for DNA and/or AdDNV analysis: American Cricket Ranch (Bill Wright), Ghann's Cricket Farm (Clay Ghann), California Cricket Ranch (Mark Eaker), Rainbow Mealworms (Betty Rhyme), Bassett's Cricket Farm (Russell Bassett), Hatley Cricket Ranch (Cynthia Hatley), Hurst-Young Wholesale Bait (Mike Young), The Gourmet Rodent (Bill Brant) , Monkfield Nutrition, Ltd. (Jo Wise), and Top Hat Crickets (Dave and Bob Eldred) .

Special thanks to Mark Eaker for paying for shipping costs of live material from England and personally bringing them to DBW. Clay Ghann, President of the CHIRP group, provided useful discussions. Russell Bassett of Bassett's Cricket Farm provided early encouragement. Howard Labe of Pets & More in Campbell, CA, initially peaked our interest.

Field assistance was provided by David C. Lightfoot, Vincent F. Lee, Brian I. Weissman, and Daniel W. Weissman.

Consultation and advice were provided by Thomas J. Walker, Scott K. Sakaluk, Anthony Zera, and Daniel Simberloff. Luciano de Pinho Martins verified that "Species C" crickets of David *et al.* (2003) agree with *Gryllus argentinus* of Martins & Zefa (2011).

Vincent F. Lee helped obtain literature. Daniel W. Weissman took all specimen photographs. David C.F. Rentz looked for commercial crickets available in Queensland, Australia.

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FIGURE 8. Joint Ghann's Cricket Farm – Top Hat Cricket Farm ad from May, 2012, issue of *Reptiles*, a trade magazine, promoting USDA authorized *G. assimilis* over illegal *G. locorojo*.

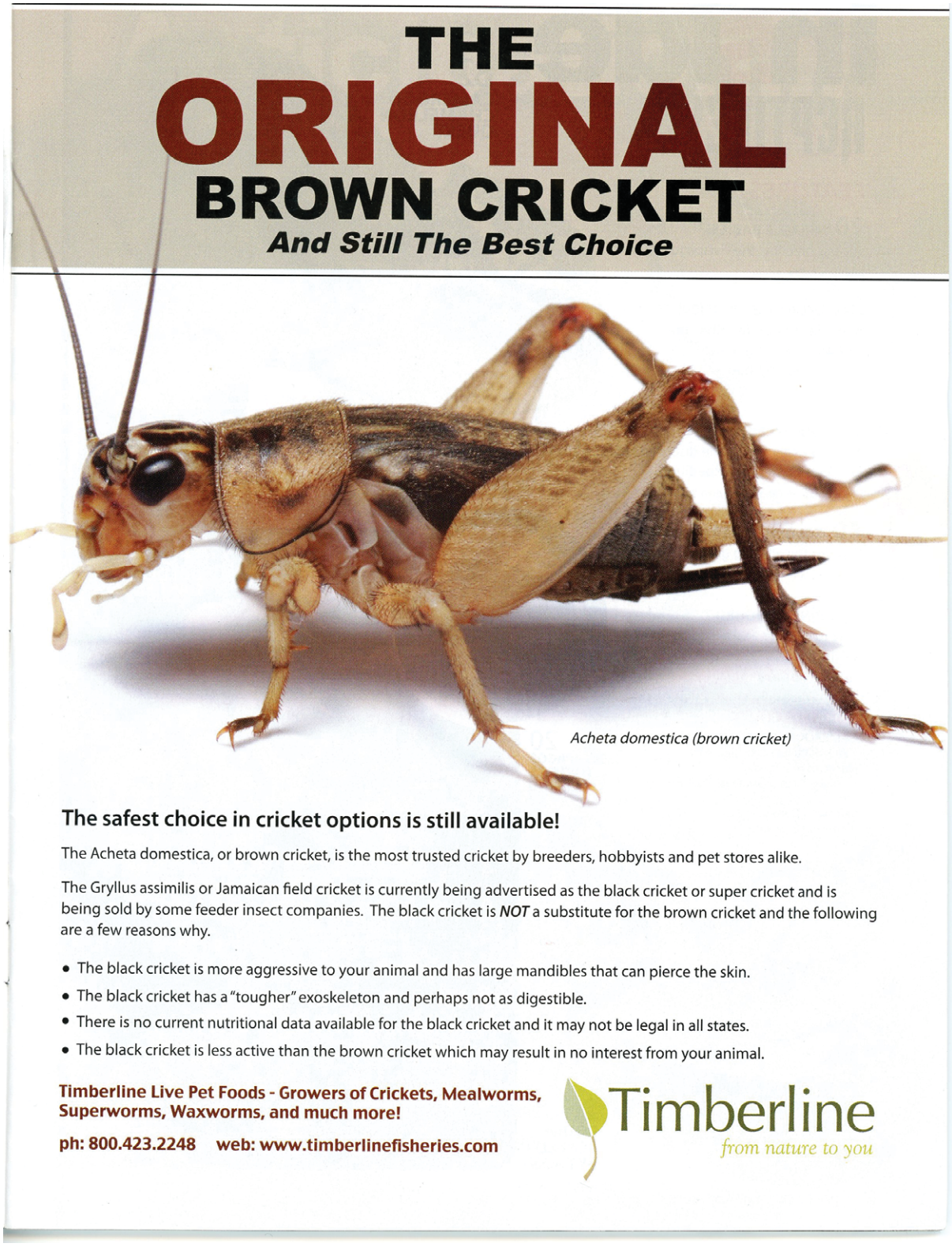

FIGURE 9. Timberline Live Pet Foods ad from May, 2012, issue of *Reptiles*, a trade magazine, promoting the advantages of *A. domestica* (sic) over other available crickets.

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APPENDIX A. Repository, identification authority, GenBank accession number, and specimen collection data for our specimens mapped in Fig. 6.

SPECIMEN ID IN TREE (Fig. 6)	SPECIMEN REPOSITORY	IDENTIFICATION AUTHORITY	GENBANK ACCESSION No.	SPECIMEN COLLECTION DATA
G6	CAS	DBW	JX269077	USA, California, San Benito Co., Lone Tree Rd. 6.5 m SE Fairview Rd. 1480' 21-iii-2003. N36° 54.53' W121° 17.09'. DBW
G61	CAS	DBW	JX269069	USA, Florida, Alachua Co., Gainesville, 23-viii-2003. T.J. Walker
G62	CAS	DBW	JX269070	USA, Florida, Alachua Co., Gainesville, 23-viii-2003. T.J. Walker
G85	CAS	DBW	JX269094	Mexico, Coahuila, Cuatrocienegas - O'Campo Rd. (Hwy 111) 1.3 km NW Cuatrocienegas Plaza, 2600' 22-ix-2003. DBW
G152	CAS	DBW	JX269073	USA, Utah, Washington Co., Zion National Park, Zion Museum. 3980' 19-v-2001. DBW
G153	CAS	DBW	JX269074	USA, Utah, Washington Co., Zion National Park, Zion Museum. 3980' 19-v-2001. DBW
G204	CAS	DBW	JX269075	USA, California, Los Angeles Co., Cal State Northridge, early April, 2004. DAG
G205	CAS	DBW	JX269076	USA, California, Los Angeles Co., Cal State Northridge, early April, 2004. DAG
G248	CAS	DBW	JX269054	USA, California, Santa Barbara Co., Santa Cruz Island, 11-vii-2004. DBW
G251	CAS	DBW	JX269055	USA, California, Santa Barbara Co., Santa Cruz Island, 11-vii-2004. DBW
G624	CAS	DBW	JX269071	USA, California, Santa Clara Co., Los Gatos, 12-viii-2006. DBW
G625	CAS	DBW	JX269072	USA, California, Santa Clara Co., Los Gatos, 12-viii-2006. DBW
G709	CAS	DBW	JX269067	USA, Vermont, Addison Co., Middlebury, 5-x-2008. A. Weissman
G710	CAS	DBW	JX269068	USA, Vermont, Addison Co., Middlebury, 5-x-2008. A. Weissman
G1303	CAS	DBW	JX269064	USA, California, Kern Co., 7 m N Hwy 138 on Techachapi Willow Springs Rd. 3520' 28-v-2009. N35° 02' 0.6" W118° 21' 6.9". DBW
G1323	CAS	DBW	JX269065	USA, California, Riverside Co., Pinyon Flats - Hwy 74 intersection with Pinon Dr. 3800' 26-v-2009. N33° 34' 58.8" W116° 27" 22.9". DBW
G1324	CAS	DBW	JX269078	USA, California, Tulare Co., Hwy 190 10.5 m W Springville. 700' 29-v-2009. N36° 3' 15.4" W118 ° 55' 5.2". DBW
G1344	CAS	DBW	JX269057	USA, Oklahoma, Texas Co., Guymon. 3380' 1-vii-2009. DBW
G1347	CAS	DBW	JX269056	USA, Texas, Howard Co., Big Springs VA Hospital. 2880' 30-vi-2009. DBW
G1462	CAS	DBW	JX269063	Mexico, Chihuahua, 24 km W Janos. 4576' 13-ix-2009. N30° 53' 55.73" W108° 26' 18.25" D.C. Lightfoot
G1463	CAS	DBW	JX269062	Mexico, Chihuahua, 24 km W Janos. 4576' 13-ix-2009. N30° 53' 55.73" W108° 26' 18.25" D.C. Lightfoot
G1901	CAS	DBW	JX269052	USA, Texas, Val Verde Co., Del Rio. 1140' 7-ix-2010. DBW
G1902	CAS	DBW	JX269053	USA, Texas, Kinney Co., Bracketville, 1160' 7-ix-2010. N29° 18' 29.7 W100° 24' 50.4. DBW
G2019	CAS	DBW	JX269059	USA, Texas, Jefferson Co., Sabine Pass. 20' 10-vi-2011. N29° 43' 36.3" W93° 53' 57.0" DBW

continued next page

APPENDIX A. (continued)

SPECIMEN ID IN TREE (Fig. 6)	SPECIMEN REPOSITORY	IDENTIFICATION AUTHORITY	GENBANK ACCESSION No.	SPECIMEN COLLECTION DATA
G2031	CAS	DBW	JX269058	USA, Texas, Jefferson Co., Sea Rim State Park, sea level, 10-vi-2011. N29° 40' 5.6" W94° 4' 16.3". DBW
G2151	CAS	DBW	JX269091	South Africa, Mpulanga Province, Sabi Sands Game Reserve, 170 km NE Nelspruit, Arathusa Safari Lodge, 980', 7-viii-2011. DBW
G2152	CAS	DBW	JX269092	same as G2151
G2155	CAS	DBW	JX269093	same as G2151
G2159	CAS	DBW	JX269034	USA, California, Santa Clara Co., Campbell, Pets & More Pet Store. 20-ix-2011. H. Labe
G2160	CAS	DBW	JX269035	same as G2159
G2191	CAS	DBW	JX269022	USA, Georgia, Richmond Co., Augusta, Ghann's Cricket Farm. xi-2011. C. Ghann
G2192	CAS	DBW	JX269023	same as G2191
G2193	CAS	DBW	JX269024	same as G2191
G2194	CAS	DBW	JX269025	same as G2191
G2195	CAS	DBW	JX269026	USA, California, Tulare Co., Visalia, Bassett's Cricket farm, 15-x-2011. R. Bassett
G2196	CAS	DBW	JX269027	same as G2195
G2197	CAS	DBW	JX269028	same as G2195
G2198	CAS	DBW	JX269029	same as G2195
G2199	CAS	DBW	JX269030	USA, California, San Diego Co., Lakeside, American Cricket Ranch, 10-xi-2011. B. Wright
G2200	CAS	DBW	JX269031	same as G2199
G2201	CAS	DBW	JX269032	same as G2199
G2202	CAS	DBW	JX269033	same as G2199
G2216	CAS	DBW	JX269043	USA, California, Los Angeles Co., Compton, Rainbow Mealworms, 13-xii-2011. F. Rhyme
G2217	CAS	DBW	JX269044	same as G2216
G2218	CAS	DBW	JX269045	same as G2216
G2219	CAS	DBW	JX269046	same as G2216
G2220	CAS	DBW	JX269047	Denmark, Copenhagen, 10-i-2012. T. Valentin
G2221	CAS	DBW	JX269048	same as G2220
G2222	CAS	DBW	JX269049	same as G2220
G2225	CAS	DBW	JX269082	France, Lorraine, Moselle, Terville, Procanis Pet Store, 14-iv-2012. Crickets from WWW.Bugs-International.com, Germany.
G2226	CAS	DBW	JX269083	same as G2225

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APPENDIX A. (continued)

SPECIMEN ID IN TREE (Fig. 6)	SPECIMEN REPOSITORY	IDENTIFICATION AUTHORITY	GENBANK ACCESSION No.	SPECIMEN COLLECTION DATA
G2227	CAS	DBW	JX269084	same as G2225
G2229	CAS	DBW	JX269086	same as G2228
G2229	CAS	DBW	JX269086	same as G2228
G2230	CAS	DBW	JX269087	same as G2228
G2231	CAS	DBW	JX269037	same as G2228
G2232	CAS	DBW	JX269038	same as G2228
G2333	CAS	DBW	JX269039	same as G2228
G2234	CAS	DBW	JX269040	same as G2228
G2235	CAS	DBW	JX269041	same as G2228
G2236	CAS	DBW	JX269042	same as G2228
G2237	CAS	DBW	JX269088	USA, California, San Diego Co., Pet Kingdom, 10-v-2012. M. Eaker
G2238	CAS	DBW	JX269089	same as G2237
G2239	CAS	DBW	JX269090	same as G2237
LM1			JX269050	Brazil, Rio Grande Do Sul, Pelotas, L. Martins
LM2			JX269051	same as LM1
TJW 3			JX269060	USA, Florida, Monroe Co., Key Largo 23-viii-1958. T. J. Walker
TJW 5			JX269061	USA, Florida, Miami-Dade Co., Long Pine Key 22-23-ix-1980. T. J. Walker
VV1			JX269036	Russia, Moscow, Moscow Zoo, 2010. V. Vedenina
WG5			JX269066	USA, Florida, Nassua Co., Yulee, as nymph 22-vii-2002. D. A. Gray
WG22			JX269081	W. H. Cade culture, originally from Harare, Zimbabwe, Africa
WG23			JX269080	same as WG22
2006-305			JX269095	S. E. Walker culture, from Gerald Pollack culture, originally from Queensland, Australia
2006-306			JX269096	same as 2006-305
2009-018			JX269079	T. Tregenza culture, originally from Oviedo, Asturias, Spain, x-2009.

GenBank data

AF248686	Germany
AF248692	USA, Florida, Alachua Co., Gainesville
EU557269	Locality not in GenBank, associated publication in Chinese: Ye et al. (2008)