

**DETECTION AND IDENTIFICATION OF ALIEN DNA IN MUSEUM SPECIMENS OF
HETEROPTERA USING MOLECULAR TECHNIQUES – A POSSIBILITY FOR APPLYING IN
FORENSIC ENTOMOLOGY***

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ABSTRACT: Studies on nuclear DNA of museum specimens of pentatomoid bugs (Cydnidae, Dinidoridae, Thyreocoridae, and Tessaratomidae) are presented. Sequences of nuclear 28S rDNA subunit were analysed in the aspect of its usefulness in forensic entomology. Results of the study demonstrated that microorganisms and parasites detected by PCR methods can be useful in determining the geographical origin of the host-species with degraded DNA.

KEY WORDS: forensic entomology, nuclear DNA, 28S rDNA, Heteroptera, Pentatomoidea, museum specimens, alien DNA.

Introduction

In last two decades, molecular techniques have been applied in many fields of science, such as phylogenetics, biogeography, medicine or forensic investigations. Nowadays, the analysis of DNA extracted from biological traces is widely used, especially in modern forensic investigations, where a molecular approach to identification of both, victims and criminals, are used to a large extent. Nuclear DNA in particular, seems to be a significant source for such analyses; however, also mitochondrial markers showed good feasibilities in human or animal species identification (Bellis et al. 2003, Caenazzo et al. 2008, Lis et al. 2011).

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Application of DNA obtained from insects found at a crime scene can be regarded as the entirely new approach in forensic entomology. Since the late 19th century, insects are used to estimate PMI (post mortem interval) in homicide and other investigations. Most recently, many studies have demonstrated that DNA extracted from insect tissues can provide more informative evidences for investigations. In literature, there are numerous papers describing cases when a variety of blood feeding insects were used even to obtain human DNA (e.g., Wells et al. 2001, Spitaleri et al. 2006, Szalanski et al. 2006, Bucheli et al. 2010, Kester et al. 2010, Rasmy 2011).

However, there are no papers which present the utility of alien DNA, obtained from the insect tissues, in forensic investigations. Therefore, in this paper the usefulness of alien DNA for such studies is considered.

Material and methods

17 samples from museum specimens of four families of pentatomoid bugs (Cydnidae, Dinidoridae, Thyreocoridae, Tessaratomidae – Table 1) were selected for nuclear DNA analyses (one region of nDNA, i.e., 28S rDNA was analysed). All specimens come from the Heteroptera collection at the Department of Biosystematics (Opole University, Poland) (Lis et al. 2011).

DNA extraction, purification and amplification were performed at the Centre for Biodiversity Studies (Department of Biosystematics, Opole University, Poland) using techniques described by Lis et al. (2011).

Two primer sequences, i.e., 28Sa and 28Sb, were used for PCR amplification (Whiting et al. 1997, Edgecombe et al. 2002.). Sequencing was conducted at the Health Care Centre GENOMED (Warsaw, Poland). The trace files (electropherograms) produced by the automated DNA sequencers were edited by the *Trace Data File Editor* – Figs 1-8).

For identification of alien DNA found in studied insects, obtained sequences were sent to the Web Browser for conducting BLAST searches (using *blastn* on NCBI).

Results

Readable sequences were obtained from all studied specimens. Unfortunately, no traces of original species DNA were recovered (all obtained sequences represented alien DNA). Sequences length varies from 60 bp to 338 bp (see Table 2, and Figures 1-8), and, usually, are characterized by a high level of noises.

In five cases (see Table 2) obtained sequences suggested similarities of studied DNA to DNA of *Saccoglossus kowalevskii* (Hemichordata), seven sequences suggested similarities to fungi, two – to species of Cryptosporidiidae (Apicomplexa), another two – to species of *Drosophila* (Diptera), and one – to *Nematostella vectensis* (Cnidaria). No similarity of the recovered sequence to any species from GenBank was found only for *Strombosoma impictum* (Thyreocoridae), most probably because of its sequence length (60 bp) and high level of degradation.

Discussion

Fungi. Though seven obtained sequences of alien DNA suggested close similarities to fungi species (Table 2), in four cases more than a single result were received from GenBank for each entry, i.e., for *Ochetostethus balcanicus*, *Coridius laosanus*, *Megymenum paralleum*, and *Galgupha difficilis*. Such situation may be caused by unclear systematic position of many fungi species, e.g., resulted *Lodderomyces elongisporus* can be a sexual stage of *Candida parapsilosis*, which is closely related to *C. tropicalis* (James et al. 1994).

All fungi species, for which DNA was sequenced, are distributed worldwide (according to the Global Biodiversity Information Facility [GBIF] website). It seems possible that in all cases this alien DNA originated from a contact with man (i.e., *Malassezia globosa*, *Ajellomyces dermatidis*, *Penicillium marneffei* – suggesting human skin diseases; *Candida tropicalis* – usually occurring on human hands), or, was acquired from host plants during feeding process (*Phaeosphaeria nodorum*, *Ashbya gossypii*, *Mycosphaerella punctiformis*, *Botryosphaeria dothidea* – all are plant pathogens) (Smith and Stanosz 2001, Verkley 2004, Phillips et al. 2005, Hospenthal and Rinaldi 2008, Kirk et al. 2008, Blixt 2009).

Apicomplexa. Two pathogenic protists, i.e., *Cryptosporidium hominis* and *C. parvum* were detected in *Peltoxys sataranus* and *Coridius laosanus* (Table 2). Both protist species are human parasites; moreover, *C. parvum*, is also pathogenic to cattle (Hashim et al. 2006). Most probably this alien DNA originated from a contact with man, because studied specimens of both heteropterans come from India and China, where both pathogens are injurious to people (Hashim et al. 2006).

Cnidaria. In one case (i.e., *Coridius nepalensis*), results of our DNA study show similarities to DNA of *Nematostella vectensis* (Cnidaria). According to the Global Biodiversity Information Facility [GBIF] website, this cnidarian species of the family *Edwardsiidae*, is distributed from eastern coasts of the United States to the southern coasts of Great Britain. Because, the similarity of DNA sequences is relatively low (84%) and concerns only 195 bp (out of the total 288 bp) we assume this result as totally unbelievable, but we can't give a sound explanation for it (see also: Putnam et al. 2007).

Diptera. Also, in *Tessarotoma quadrata*, results of the DNA study was ambiguous (see Table 2), i.e., 100% similarity, but only on the length of 79 bp (out of the total 325 bp). Most probably, the presence of DNA of other (parasitic) dipteran species, which DNA was never before sequenced (and therefore not present in GenBank), is the only explanation.

Hemichordata. In five cases (Table 2) obtained results indicated a similarity of alien DNA to DNA of *Saccoglossus kowalevskii* (class Enteropneusta). Because, it is impossible to find this species inside the insect body, we assume this fact as a result of a contamination by human DNA (see also: Acorn Worm Genome Project, www.hgsc.bcm.edu/content/acorn-worm-genome-project).

Conclusions

In forensic entomology, when a crime has been committed, most often a murder, insects around and on the victim's body become the very important evidence. The precise DNA sequence of insects that appear on a corpse has been carefully documented by forensic entomologists.

Such data are usually utilized for estimation of post-mortem interval (PMI), to locate scenes of murder crimes, and for toxicological analyses in the absence of human tissues and fluids normally taken for such purposes.

Results of the present study have additionally demonstrated the potential utility of insects as good samples of alien DNA, which can be utilized for forensic purposes. The presence of alien DNA (e.g., DNA of fungi, or parasite DNA) in insect tissues may provide helpful information for resolving the problem of geographic origin of forensically important samples; as the most important this can be used to locate scenes of murder crimes.

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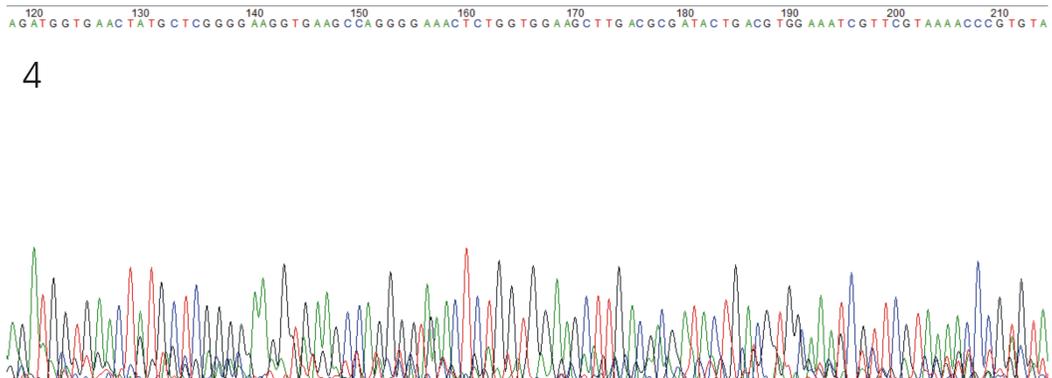
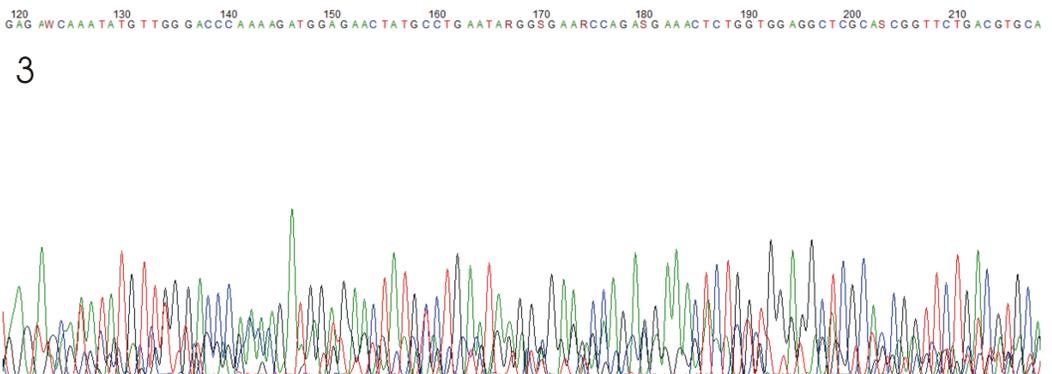
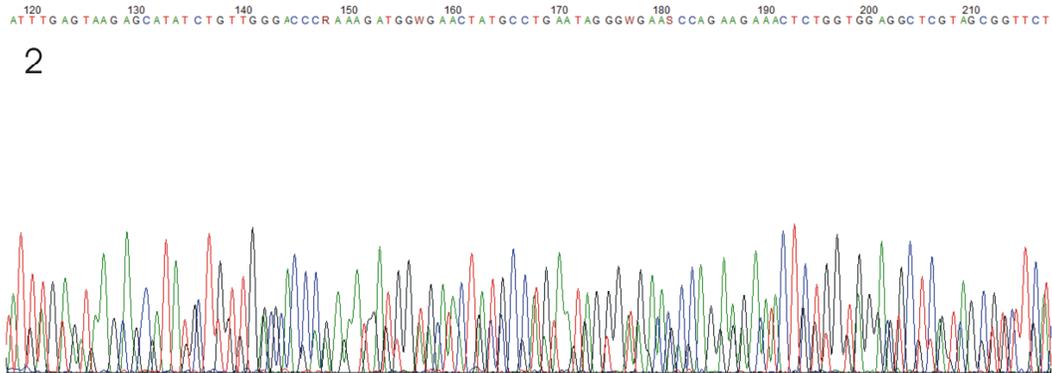
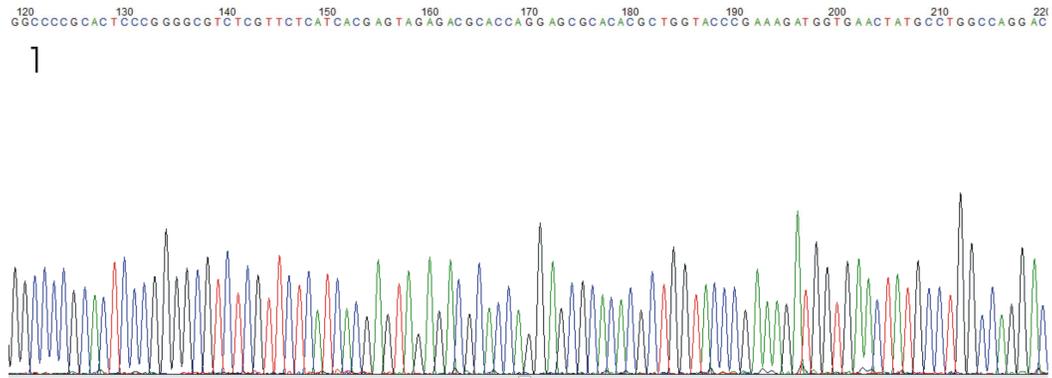
Table 1. Details of examined specimens.

Species and family	Geographic origin	Year collected
<i>Adrisa flavomarginata</i> (Voll.) Cydnidae	New Caledonia	2008
<i>Adrisa flavomarginata</i> (Voll.) Cydnidae	New Caledonia	2008
<i>Chilocoris assmuthi</i> Bredd. Cydnidae	Pakistan	1972
<i>Ochetostethus balcanicus</i> Wagn. Cydnidae	Croatia	1957
<i>Peltoxys sataranus</i> Lis & Lis Cydnidae	India	2002
<i>Pseudoscoparipes nilgiricus</i> Lis Cydnidae	India	1976
<i>Coridius laosanus</i> (Dist.) Dinidoridae	China	1915
<i>Coridius nepalensis</i> (Westw.) Dinidoridae	Thailand	1995
<i>Coridius prolixus</i> (Leth.) Dinidoridae	Togo	1954
<i>Cyclopelta obscura</i> (Leth. & Serv.) Dinidoridae	Thailand	1995
<i>Cyclopelta siccifolia</i> (Westw.) Dinidoridae	Nepal	1986
<i>Cyclopelta siccifolia</i> (Westw.) Dinidoridae	Sri Lanka	1894
<i>Megymenum brevicorne</i> (F.) Dinidoridae	Thailand	1995
<i>Megymenum paralleum</i> Voll. Dinidoridae	Thailand	1995
<i>Tessarotoma quadrata</i> Dist. Tessaratomidae	Vietnam	1990
<i>Galgupha difficilis</i> (Bredd.) Thyreocoridae	Brasil	1975
<i>Strombosoma impictum</i> (Stål) Thyreocoridae	Zaire	1932

Table 2. Similarities of obtained sequences to those available from GenBank (using BLAST); (length of the sequence and similarity percentage for each species are shown in square brackets).

Studied species Family [length of sequence in bp]	Highest similarity to species from GenBank
<i>Adrisa flavomarginata</i> Cydnidae [338]	<i>Saccoglossus kowalevskii</i> [93% (311)] Harrimaniidae (Hemichordata)
<i>Adrisa flavomarginata</i> Cydnidae [338]	<i>Saccoglossus kowalevskii</i> [93% (311)] Harrimaniidae (Hemichordata)
<i>Chilocoris assmuthi</i> Cydnidae [335]	<i>Saccoglossus kowalevskii</i> [92% (305)] Harrimaniidae (Hemichordata)
<i>Ochetostethus balcanicus</i> Cydnidae [295]	<i>Phaeosphaeria nodorum</i> [92% (411)] Phaeosphaeriaceae (Fungi: Dothideomycetes)
	<i>Lodderomyces elongisporus</i> [92% (411)] Debaryomycetaceae (Fungi: Saccharomycetes)
<i>Peltoxys sataranus</i> Cydnidae [292]	<i>Cryptosporidium hominis</i> [95% (279)] Cryptosporidiidae (Apicomplexa: Eucoccidiorida)
<i>Pseudoscoparipes nilgiricus</i> Cydnidae [291]	<i>Malassezia globosa</i> [89% (357)] Malasseziaceae (Fungi: Exobasidiomycetes)
<i>Coridius laosanus</i> Dinidoridae [287]	<i>Cryptosporidium hominis</i> [88% (183)] Cryptosporidiidae (Apicomplexa: Eucoccidiorida)
	<i>Cryptosporidium parvum</i> [88% (183)] Cryptosporidiidae (Apicomplexa: Eucoccidiorida)
<i>Coridius nepalensis</i> Dinidoridae [288]	<i>Nematostella vectensis</i> [84% (195)] Edwardsiidae (Cnidaria: Actiniaria)
<i>Coridius prolixus</i> Dinidoridae [288]	<i>Malassezia globosa</i> [81% (237)] Malasseziaceae (Fungi: Exobasidiomycetes)
<i>Cyclopelta obscura</i> Dinidoridae [330]	<i>Saccoglossus kowalevskii</i> [92% (305)] Harrimaniidae (Hemichordata)
<i>Cyclopelta siccifolia</i> Dinidoridae [290]	<i>Mycosphaerella punctiformis</i> [87% (327)] Mycosphaerellaceae (Fungi: Dothideomycetes)
<i>Cyclopelta siccifolia</i> Dinidoridae [293]	<i>Botryosphaeria dothidea</i> [85% (313)] Botryosphaeriaceae (Fungi: Dothideomycetes)
<i>Megymenum brevicorne</i> Dinidoridae [337]	<i>Saccoglossus kowalevskii</i> [91% (302)] Harrimaniidae (Hemichordata)

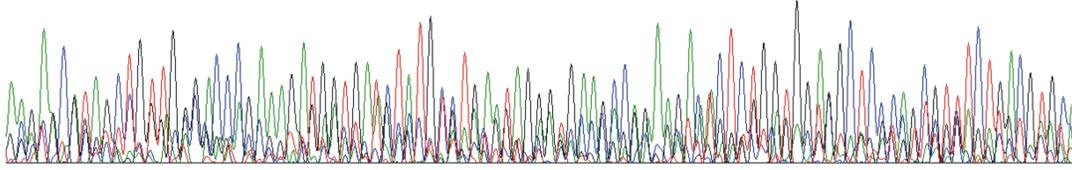
<i>Megymenum paralleum</i> Dinidoridae [326]	<i>Talaromyces stipitatus</i> [88% (361)] Trichocomaceae (Fungi: Eurotiomycetes)
	<i>Penicillium marneffeii</i> [88% (361)] Trichocomaceae (Fungi: Eurotiomycetes)
	<i>Ajellomyces dermatitidis</i> [88% (361)] Ajellomycetaceae (Fungi: Eurotiomycetes)
<i>Galgupha difficilis</i> Thyreocoridae [290]	<i>Candida tropicalis</i> [84% (315)] Incertae sedis (Fungi: Saccharomycetes)
	<i>Penicillium marneffeii</i> [84% (315)] Trichocomaceae (Fungi: Eurotiomycetes)
	<i>Ashbya gossypii</i> [84% (315)] Saccharomycetaceae (Fungi: Saccharomycetes)
<i>Strombosoma impictum</i> Thyreocoridae [60]	No similarity found
<i>Tessarotoma quadrata</i> Tessaratomidae [325]	<i>Drosophila simulans</i> [100% (79)] Drosophilidae (Diptera)
	<i>Drosophila melanogaster</i> [100% (79)] Drosophilidae (Diptera)



Figures 1-4. Electropherograms showing the same fragment of selected sequences. 1 – *Adrisa flavomarginata* (Cydnidae), 2 – *Ochetostethus balcanicus* (Cydnidae), 3 – *Pseudoscoparipes nilgiricus* (Cydnidae), 4 – *Coridius laosanus* (Dinidoridae).

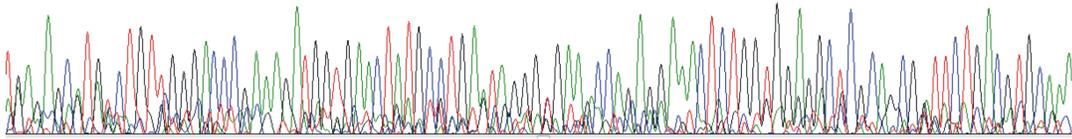
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5



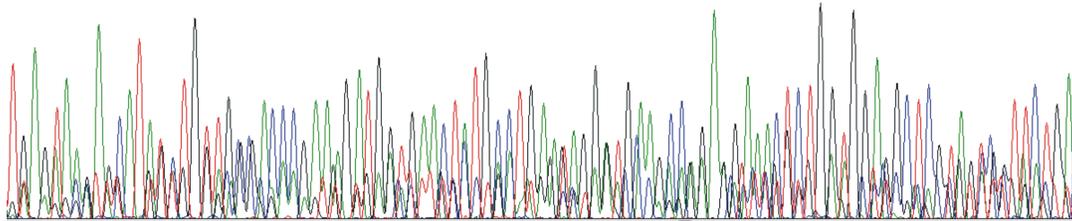
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6



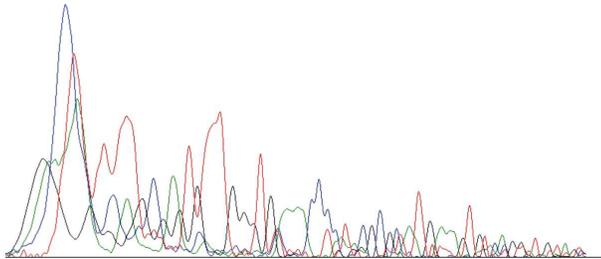
120 130 140 150 160 170 180 190 200 210
 TGAGTAASAKCATAKYTGTTGSGACCCGAAAGATGGTGAACATATGCTGAAKAGGRTGAAAGCCRGARGAAACTCTGGTGGAGKGC TCGTAKYSSTCTGAC

7



10 20 30 40 50 60
 TT GACYAGTCTTCCGAGCTTGGGTGYAACCYMTRCGCYATGAAGTCTCAGKATGTAAG

8



Figures 5-8. Electropherograms showing the fragment of selected sequences. 5 – *Coridius nepalensis* (Dinidoridae), 6 – *Megymenum paralleum* (Dinidoridae), 7 – *Galgupha difficilis* (Thyreocoridae), 8 – *Strombosoma impictum* (Thyreocoridae).