Russian Entomol. J. 15(4): 427–432

DNA-based identification of Lepidoptera larvae and plant meals from their gut contents

Основанное на ДНК определение гусениц Lepidoptera и растительной пищи из их кишечника

Michael A. Miller[1], Günter C. Müller[2], Vasiliy D. Kravchenko[3], Amy Junnila[4], Kim K. Vernon[5], Carney D. Matheson[6] & Axel Hausmann[1]

Михаэль А. Миллер[1], Гюнтер К. Мюллер[2], Василий Д. Кравченко[3], Эйми Джаннила[4], Ким К. Вернон[5], Кэрни Д. Матесон[6] и Аксель Хаусманн[1]

[1] Zoologische Staatssammlung München, AG DNA-TAX, Münchhausenstr. 21, D-81247 München, Germany (corresponding author, e-mail: miller@zsm.mwn.de);

[2] Department of Parasitology, The Hebrew University, Hadassah Medical School, P.O. Box 12272, Ein Kerem, Jerusalem 91120 Israel;

[3] Department of Zoology, Tel Aviv University, Tel Aviv 69978 Israel;

[4] Department of Parasitology, McGill University, Macdonald College, 21,111 Lakeshore Rd., Sainte-Anne-de Bellevue, Montreal West, H9X 3V9 Quebec, Canada;

[5] Department of Zoology, University of Queensland, Brisbane QLD 4072 Australia;

[6] Paleo-DNA Laboratory, Lakehead University, 1294 Balmoral St, Thunder Bay, P7B 5Z5 Ontario, Canada.

[1] Государственный зоологический музей Мюнхена, AG DNA-TAX, Мюнгаузен Штрассе 21, D-81247 Мюнхен, Германия (контактный адрес, e-mail: miller@zsm.mwn.de);

[2] Кафедра паразитологии, Еврейский Университет, Медицинская Школа Хадассах, почтовый ящик 12272, Эйн Керем, Иерусалим 91120, Израиль;

[3] Кафедра зоологии, Университет Тель-Авива, Тель-Авив 69978, Израиль;

[4] Кафедра паразитологии, Университет МакГилла, МакДональд Колледж, 21, 111 Лайкшоэ Роуд, Св. Анна де Bellevue, Западный Монраель, H9X 3V9 Квебек, Канада;

[5] Кафедра зоологии, Университет Квинсленда, Брисбен QLD 4072, Австралия;

[6] Лаборатория палео-ДНК, Университет Лайкхеда, 1294 Балморал Стрит, Тундер Бэй, P7B 5Z5 Онтарио, Канада.

KEY WORDS: Species identification, Lepidoptera, geometrid moth larvae, *Chiasmia aestimaria*, *Eupithecia ultimaria*, host plant, DNA taxonomy, DNA barcoding, mtDNA, COI.

КЛЮЧЕВЫЕ СЛОВА: Определение вида, Lepidoptera, гусеницы пядениц, *Chiasmia aestimaria*, *Eupithecia ultimaria*, кормовое растение, таксономия ДНК, бар-кодирование ДНК, митохондриальная ДНК (mtDNA), подотдел I цитохромной оксидазы (COI).

ABSTRACT. Identification systems based on DNA (DNA barcodes) can potentially facilitate both the identification of known species and the discovery of new ones. In this study, the use of molecular markers to identify unknown immature lepidopteran stages was useful and reliable. We identified here five Israeli geometrid moth larvae as *Chiasmia aestimaria* (Hübner, 1809) using both a fragment of, and the whole, mitochondrial gene cytochrome oxidase subunit I (COI). In addition, molecular analysis of a fragment of the chloroplast rbcL gene in the gut contents revealed sequence homology with the suspected host-plant *Tamarix nilotica* (Ehrenb.). These data suggest that, in some instances, even short DNA fragments are sufficient for identifying closely related taxa to the species level.

РЕЗЮМЕ. Системы определения на основе ДНК (бар-кодирование ДНК) потенциально полезны как в идентификации известных, так и в открытии новых видов. В данном исследовании использование молекулярных маркеров оказалось полезным и надёжным. Здесь с помощью фрагмента и целого митохондриального гена подотдела I цитохромной оксидазы (COI) пять гусениц пядениц из Израиля определены как принадлежащие виду *Chiasmia aestimaria* (Hübner, 1809). Кроме того, молекулярный анализ фрагмента гена *rbc*L хлоропласта в содержимом их кишечника выявил гомологию с предполагавшимся прежде в качестве кормового растения тамариском *Tamarix nilotica* (Ehrenb.). Эти данные предполагают, что иногда даже небольших фрагментов ДНК

достаточно, чтобы определить близкородственные таксоны до видового уровня.

Introduction

A fundamental prerequisite for the study of insects and their interactions with the environment is the correct determination of species according to the currently accepted principals of taxonomy. Equally important is the accurate identification of insect food sources and feeding habits.

That being said, entomological studies can be problematic because insect larvae cannot be identified to the species level without being reared to the adult stage. In addition, the feeding behaviour of insects, particularly larvae, has been a difficult ecological interaction to study. In most insect species the identification of plant remains is complex, requiring dissections and stains, dyes or biochemical techniques [Schlein & Jacobson, 1994; Schlein & Muller, 1995]. These techniques tend to require relatively large amounts of gut material, ruling out detailed analysis of larval food, and are not too specific.

Taken together, collection of these data can provide information critical to the control of agricultural pests and insects of medical importance, as well as ensure a broader understanding of insect taxonomy.

An initiative to generate DNA sequences for all named species on the planet has led to the development of DNA taxonomy [Tautz et al., 2002, 2003] or DNA barcoding [Hebert et al., 2003, 2005]. These are diagnostic techniques, in which short DNA sequences can be used for species identification (for reviews see DeSalle et al. [2005] and Savolainen et al. [2005]). Although still controversial, the scientific benefits of DNA barcoding: (1) enable species identification of any life stage or body part, (2) facilitate species discoveries based on analyses of gene sequences, (3) promote the development of biodiversity inventories and (4) provide insights into the diversity of life in a given ecosystem.

Here we ask whether DNA in this study, identified by barcoding, is consistent with the morphological species identification for both the insect larvae and the plant meals from them. In this study, five Israeli geometrid moth larvae were identified as *Chiasmia aestimaria* (Hübner, 1809) using the mitochondrial gene cytochrome oxidase subunit I (COI). In addition, molecular analysis of a fragment of the chloroplast *rbc*L gene in the gut contents revealed sequence homology with the suspected host-plant *Tamarix nilotica* (Ehrenb.).

Material and methods

Lepidopteran larvae found feeding on an unidentified species of *Tamarix* were collected in the Jordan Valley, Israel in December 2002. The live specimens were stored in sterile 2ml cryovials filled with pure ethanol and kept at room temperature. For dissection, the larvae were put on a piece of Parafilm and opened with sterilized watchmaker forceps and micro scissors under a stereo microscope in a DNA-free UV safety cabinet (KOJAIR). The gut was separated and stored in ethanol, muscle tissue was extracted and transferred to sterile 1.5 ml Eppendorf tubes and allowed to air dry. Afterwards, the tissue was ground with glass beads and sterile pestles.

Adult Lepidoptera were chosen from a collection of geometrid voucher specimens (Zoologische Staatssammlung München). The selection was primarily focused on two geometrid species known to feed on *Tamarix* spp. [Krüger, 2001; Mironov, 2003], secondly also on their most closely related sister taxa known to occur in the Jordan Valley [Hausmann, 1991; Halperin & Sauter, 1992]. Therefore, we reduced our set of identification key reference samples to those of *C. aestimaria*, *C. syriacaria* (Staudinger, 1871) (subfamily Ennominae), *Eupithecia ultimaria* Boisduval, 1840, and *E. opistographata* (Dietze, 1906) (subfamily Larentiinae). The samples were dried specimens treated as described by Knölke et al. [2005]. DNA lysis was performed on either abdomina or single legs. For further details concerning the specimens, see Table 1.

DNA was extracted with the QIAGEN DNA tissue kit, following the manufacturer's protocol. PCR was per-

Table 1. Species, label data, length of sequence, ID-numbers and reference sequence.
Таблица 1. Виды, данные этикеток, длина последовательности, идентификационные номера и опорная последовательность.

Species	Locality	bps	DNATAX-ID	EMBL/ Gen Bank acc. No.
E. ultimaria	Israel, Arava valley, En Zin, 100m , 5.III.2000,leg. C. Li & G. Müller	205	DNATAX01855	AM282974
E. ultimaria	Israel, Judaean desert, Wadi Kelt, En Prat, 250m, 8.III.1999,leg. C.Li & G. Müller	205	DNATAX01856	AM282975
E. opistographata	Israel, Dead Sea, En Gedi, 22.IV.1989, leg. G. Müller	205	DNATAX02202	AM282976
C. aestimaria	Israel, Judaean desert, Wadi Kelt, En Prat, 250m, 8.III.1999,leg. C.Li & G. Müller	1560	DNATAX01862	AM282979
C. aestimaria	Israel, Judaean desert, Wadi Kelt, En Prat, 250m, 8.III.1999,leg. C.Li & G. Müller	1560	DNATAX01863	AM282980
C. syriacaria	Israel, mid Jordan valley Hazafon, Tirat, 4–7.VIII. 1989, leg. G. Müller	205	DNATAX01865	AM282977
unknown larva	Israel, Jordan valley, XII.2002, leg. Kravchenko & Müller	1560	DNATAX02166	AM282981
unknown larva	Israel, Jordan valley, XII.2002, leg. Kravchenko & Müller	1560	DNATAX02167	AM282982
unknown larva	Israel, Jordan valley, XII.2002, leg. Kravchenko & Müller	1560	DNATAX02168	AM282983
unknown larva	Israel, Jordan valley, XII.2002, leg. Kravchenko & Müller	1560	DNATAX02169	AM282984
unknown larva	Israel, Jordan valley, XII.2002, leg. Kravchenko & Müller	1560	DNATAX02170	AM282985
unknown larva	Israel, Jordan valley, XII.2002, leg. Kravchenko & Müller	205	DNATAX02171	AM282978

formed with a PTC 220 DYAD thermocycler (MJ Research) in a 25 µl reaction volume, using the Expand PCR system (Roche Diagnostics) with 25 pmol of each primer (forward: mtD4Geo: 5'-cycgttaataaatttacaat-3'; reverse: mtD7rev: 5'-gggaawgctatatcwggtgctcc-3', mtD12Geo: 5'-wcctttataratggrgttta-3'), 20 pmol of dNTPs, 12.5 pmol $MgCl_2$ and 0.88 units of Taq polymerase. PCR parameters consisted of initial denaturation at 94 °C for 4 min, 45 cycles at 94 °C for 1.5 min, 48°C for 1 min, 72 °C for 1.5 min, followed by a final elongation at 72 °C for 3 min. PCR results were examined using agarose electrophoresis and visualized with ethidium bromide under ultra violet light. PCR products were purified with a MinElute PCR purification kit (QIAGEN), as suggested in the manual. PCR products were used as templates for the cycle-sequencing reaction (Big Dye Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit v1.1, Applied Biosystems) with each of the PCR primers or internal primers (see Simon et al. [1994]), respectively. Cycle parameters were as follows: initial denaturation at 94 °C, 2 min; 25 cycles at 94 °C, 20 sec; 52 °C, 10 sec; 60 °C, 4 min; with final cooling to 8 °C. The sequenced product was filtered through Sephadex-G50 fine (Fluka) packed spin columns (Amersham) to remove unincorporated dye terminators, primers, and salts, and desiccated in a speed-vac. The products were resuspended and electrophoresed on an ABI 377 XL automated sequencer. All fragments were sequenced in forward and reverse directions. The sequences were analysed manually with a Sequence Navigator v. 1.0.1 (Applied Biosystems).

The plant material from the guts of the larvae was treated according to Matheson et al. [in press]. PCR resulted in a 153 bp long fragment of the ribulose bisphosphate carboxylase gene's large subunit (*rbc*L) [Poinar et al., 1998].

Results

For DNA sequence comparisons among all specimens, a 205bp sequence of the COI was used to evaluate the chance of sequence congruence. Even in this very short dataset, the species in a genus clustered clearly, as did specimens of the same species. Tree reconstructions (PAUP* v. 4.0b10) showed clear clustering of all six caterpillar sequences within one haplotype of *C. aestimaria* (Fig. 1, Table 2).

This result was surprising as the larvae had been grouped into two 'morphospecies'. Morphological re-examination of the larvae in question confirmed that the larvae were merely different moulting stages and colour variants of the same species.

Sequence variation of morphologically identified lepidopteran larvae of *C. aestimaria* is 0.49%, while species variation ranges from 16.50% between the genera (*Eupithecia* vs. *Chiasmia*), 3.88% (*Eupithecia*, n=2) or 5.83% (*Chiasmia*, n=2) between species of the same genus, respectively, 0.97% (*E. ultimaria*, n=2), and 0.49% (*C. aestimaria*, n=8) within the same species.

In addition, we investigated the sequence variability within the complete COI gene of five lepidopteran larvae and two of the *C. aestimaria* specimens. Considering the 8-fold increase in sequence data, we expected to find between 8 and 15 variable positions within the specimens of *C. aestimaria*. Table 3 shows 10 variable sites (0.64% sequence divergence) resulting in five different haplotypes. The proportion of codon position mutations is slightly shifted from mutations of the first and third codon positions in favour of the second codon positions (10% in the first, 20%

Table 2. Variable positions. There were 25 polymorphic loci identified as follows: 4 of first (16%), 1 of second (4%) and 20 of third (80%) codon positions within the first 205 bps of COI sequence.
Таблица 2. Изменчивые локусы. Выявлено 25 полиморфных локусов: 4 из первых (16%), 1 их вторых (4%) и 20 из третьих (80%) позиций кодонов среди начальныйх фрагментов гена COI длиной 205 bps.

DNATAX-ID	species	bp	12	15	18	30	42	43	69	72	76	87	88	97	108	119	120	123	129	144	150	156	165	171	177	195	201
		cp	3	3	3	3	3	1	3	3	1	3	1	1	3	2	3	3	3	3	3	3	3	3	3	3	3
DNATAX01855	*E. ultimaria*		T	T	A	A	T	T	T	T	A	A	T	C	T	C	C	C	T	C	T	*C*	A	*C*	T	T	T
DNATAX01856	*E. ultimaria*		T	T	A	A	T	T	T	T	A	A	T	C	T	C	C	C	T	C	T	*T*	A	*T*	T	T	T
DNATAX02202	*E. opistographata*		T	T	T	G	T	C	A	T	A	A	T	C	T	C	C	C	T	C	T	T	T	T	T	T	A
DNATAX01862	*C. aestimaria*		A	T	A	A	A	T	A	A	G	T	*T*	T	T	A	T	T	C	T	T	C	A	T	C	T	A
DNATAX01863	*C. aestimaria*		**A**	**T**	**A**	**A**	**A**	**T**	**A**	**A**	**G**	**T**	***C***	**T**	**T**	**A**	**T**	**T**	**C**	**T**	**T**	**C**	**A**	**T**	**C**	**T**	**A**
DNATAX01865	*C. syriacaria*		A	C	A	G	A	T	C	A	A	T	T	T	A	A	T	C	T	T	C	T	A	T	T	C	A
DNATAX02166	unknown larva		**A**	**T**	**A**	**A**	**A**	**T**	**A**	**A**	**G**	**T**	**C**	**T**	**T**	**A**	**T**	**T**	**C**	**T**	**T**	**C**	**A**	**T**	**C**	**T**	**A**
DNATAX02167	unknown larva		**A**	**T**	**A**	**A**	**A**	**T**	**A**	**A**	**G**	**T**	**C**	**T**	**T**	**A**	**T**	**T**	**C**	**T**	**T**	**C**	**A**	**T**	**C**	**T**	**A**
DNATAX02168	unknown larva		**A**	**T**	**A**	**A**	**A**	**T**	**A**	**A**	**G**	**T**	**C**	**T**	**T**	**A**	**T**	**T**	**C**	**T**	**T**	**C**	**A**	**T**	**C**	**T**	**A**
DNATAX02169	unknown larva		**A**	**T**	**A**	**A**	**A**	**T**	**A**	**A**	**G**	**T**	**C**	**T**	**T**	**A**	**T**	**T**	**C**	**T**	**T**	**C**	**A**	**T**	**C**	**T**	**A**
DNATAX02170	unknown larva		**A**	**T**	**A**	**A**	**A**	**T**	**A**	**A**	**G**	**T**	**C**	**T**	**T**	**A**	**T**	**T**	**C**	**T**	**T**	**C**	**A**	**T**	**C**	**T**	**A**
DNATAX02171	unknown larva		**A**	**T**	**A**	**A**	**A**	**T**	**A**	**A**	**G**	**T**	**C**	**T**	**T**	**A**	**T**	**T**	**C**	**T**	**T**	**C**	**A**	**T**	**C**	**T**	**A**

Abbreviations: bp — base pair; cp — codon position. Bold text — sample that exhibit sequence homology. Italicised letters — polymorphic genetic loci within a single species.

Сокращения: bp — основная пара; cp — позиция кодона. Выделение жирным шрифтом — проба, демонстрирующая гомологию последовательности. Курсив — полиморфные локусы генов в пределах одного вида.

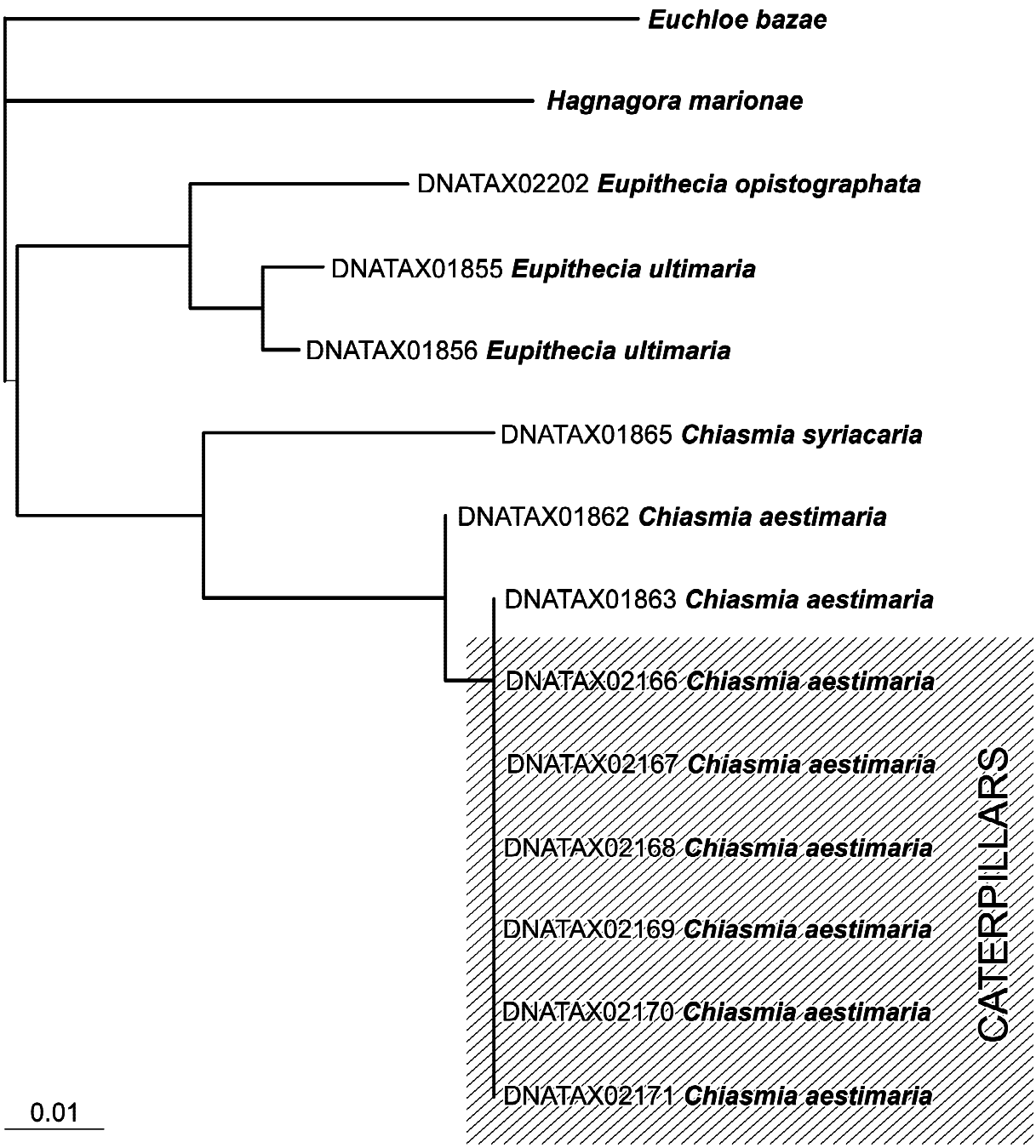

Fig. 1. Distance (UPGMA) tree of the investigated taxa generated with PAUP*. All previously unknown caterpillars cluster with one of the two haplotypes of *C. aestimaria*. Outgroup references are *Euchloe bazae* (Pieridae: Pierinae) and *Hagnagora marionae* (Geometridae: Larentiinae).

Рис. 1. Дистанционное (UPGMA) дерево всех исследованных таксонов, полученное с помощью программы PAUP*. Все ранее не идентифицированные гусеницы группируются в один из двух гаплотипов *C. aestimaria*. Внешняя группа — *Euchloe bazae* (Pieridae: Pierinae) и *Hagnagora marionae* (Geometridae: Larentiinae).

in the second, and 70% in the third codon positions). Except for pos. 1073 and 1541, the mutations have no effect on the coding protein.

The larval gut contents of plant meals were also characterised using molecular techniques. The plant material dissected from the gut of the larvae of *C. aestimaria* was extracted and sequenced. Sequence homology with a fragment of the chloroplast *rbc*L gene (153 bp) was 100% for *Tamarix nilotica* (GenBank ass. no. AY545896).

Table. 3. Variable sites of 7 specimens (2 adults and 5 larvae) of *C. aestimaria* within the complete COI gene (1530 bp) and a 30 bp fragment of the adjacent tyrosine tRNA gene. Abbreviations see in Table 2.
Таблица 3. Изменчивые места у 7 экземпляров (2 имаго и 5 гусениц) *C. aestimaria* в пределах полного гена COI (1530 bp) и фрагмента в 30 bp соседнего гена тирозина tRNA. Сокращения см. в таблице 2.

DNATAX-ID	species	bp	88	366	684	708	759	789	981	1073	1182	1541	Haplotype no.
		cp	1	3	3	3	3	3	3	2	3	2	
DNATAX01862	*C. aestimaria*		T	G	T	G	C	A	A	A	T	A	1
DNATAX01863	*C. aestimaria*		C	G	T	G	C	A	A	A	C	A	2
DNATAX02166	*C. aestimaria* larva 1		C	A	T	G	C	G	C	A	C	A	3
DNATAX02167	*C. aestimaria* larva 2		C	G	T	A	T	A	A	G	C	G	4
DNATAX02168	*C. aestimaria* larva 3		C	G	T	G	C	A	A	A	C	A	2
DNATAX02169	*C. aestimaria* larva 4		C	G	C	G	C	A	A	A	C	A	5
DNATAX02170	*C. aestimaria* larva 5		C	G	T	G	C	A	A	A	C	A	2

Discussion

Identification systems based on DNA can potentially facilitate both the identification of known species and the discovery of new ones [Janzen, 2004]. The technique of DNA barcoding is based on the premise that diversity within a short, standardized segment of the genome can provide a "biological barcode" that enables identifications at the species level.

In this study, the use of molecular markers to identify unknown immature lepidopteran stages was useful and reliable. The results suggest that, in some instances, even short DNA fragments are sufficient for identifying closely related taxa to the species level. Intra-populational sequence divergence of the COI gene of the investigated geometrid moths was below 1% (Table 2) and the sequence variation between closely related sister species was up to 10 times higher (see Hebert et al. [2005]). Therefore, DNA-based identification systems ('DNA barcodes') seem to provide a valuable tool for 'species' identification, especially if adequate databases of plant and insect reference sequences are available.

The slowly evolving *rbc*L gene does not seem to provide an unambiguous resolution of plant identity to the species level in all instances. Despite this, it can be appropriate in confirming or negating the observed identity of presumed or previously identified host-plant families. Additional molecular markers could refine this method even further in the future.

Collaboration between biological disciplines will enable the development of a taxonomic system integrating phenotypic and genotypic criteria. This will increase our understanding of biodiversity despite the limitations of time and funding [Wilson, 2004]. The first scientific initiatives like the Consortium for the Barcode of Life (CBOL, www.barcodinglife.org) have already been established to "foster and direct the development of DNA barcoding", while discussions continue.

ACKNOWLEDGEMENTS. We thank Konstantin Witt for his technical assistance and Dr. L. Schnur for revising the manuscript. This research, conducted at the Zoologische Staatssammlung in Munich, Germany, was part of the DNATAX project aimed at establishing an insect sequence database for DNA taxonomic purposes. The project is part of the Entomological Data Information System (EDIS) initiative under the direction of BIOLOG, funded by the German Federal Ministry for Education and Research (BMBF project ID 01LC99004/2).

References

DeSalle R., Egan M.G. & Siddall M. 2005. The unholy trinity: Taxonomy, species delimitation and DNA barcoding // Philos. Trans. R. Soc. Lond., B. Vol.360. No.1462. P.1905–1916.

Halperin J. & Sauter W. 1992. An annotated list with new records of Lepidoptera associated with forest and ornamental trees and shrubs in Israel // Israel J. Entom. Vols 25/26. P.105–147.

Hausmann A. 1991. Beitrag zur Geometridenfauna Palästinas: Die Spanner der Klapperich-Ausbeute aus Jordanien (Lepidoptera, Geometridae) // Mitt. Münch. Ent. Ges. Bd.81. S.111–163.

Hebert P.D.N., Cywinsky A., Ball S.L. & deWaard J.R. 2003. Biological identifications through DNA barcodes // Proc. R. Soc. Lond., B. Vol.270. No.1511. P.313–321.

Hebert P.D.N., Penton E.H., Burns J.M., Janzen D.H. & Hallwachs W. 2005. Ten species is one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator* // Proc. Natn. Acad. Sci. Vol.101. No.41. P.14812–14817.

Janzen D.H. 2004. Now is the time // Phil. Trans. R. Soc. Lond., B. Vol.359. P.731–732.

Knölke S., Erlacher S., Hausmann A., Miller M.A. & Segerer A.H. 2005. A procedure for combined genitalia dissection and DNA extraction in Lepidoptera // Insect Syst. Evol. Vol.35. No.4. P.401–409.

Krüger M. 2001. A revision of the tribe Macariini (Lepidoptera: Geometridae: Ennominae) of Africa, Madagascar and Arabia // Bull. Nat. Hist. Mus. Lond. (Entom.). Vol.70. No.1. P.1–502.

Matheson C.D., Müller G.C., Vernon K., Junnila A., Hausmann A., Miller M.A., Greenblatt C. & Schlein Y. 2006. A PCR method for detection of plant meals from the gut of insects // Organisms, Diversity and Evolution (in press).

Mironov V. 2003. Larentiinae II (Perizomini and Eupitheciini) // Hausmann A. (Ed.): The Geometrid Moths of Europe. Vol.4. P.1–463.

Poinar H. N., Hofreiter M., Spoulding W.G., Martin P.S., Stankiewicz B.A., Bland H., Evershed R.P., Possnert G. & Pääbo S. 1998. Molecular coproscopy: Dung and diet of the extinct ground sloth *Nothrotheriops shastensis* // Science. Vol.281. P.402–406.

Savolainen V., Cowan R.S., Vogler A.P., Roderick G.K., & Lane R. 2005. Towards writing the encyclopedia of life: An introduction to DNA bar-coding // Philos. Trans. R. Soc. Lond., B. Vol.360. No.1462. P.1805–1811.

Schlein Y. & Jacobson R.L. 1994. Mortality of *Leishmania major* in *Phlebotomus papatasi* caused by plant feeding of the sand flies // Am. J. Trop. Med. Hyg. Vol.50. P.20–27.

Schlein Y. & Müller G. 1995. Assessment of plant tissue feeding by sandflies (Diptera: Psychodidae) and mosquitoes (Diptera: Culicidae) // J. Med. Entom. Vol.32. P.882–887.

Simon C., Frati F., Beckenbach A., Crespi B., Liu H. & Flook P. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers // Ann. Entom. Soc. America. Vol.87. P.651–702.

Tautz D., Arctander P., Minelli A., Thomas R.H. & Vogler A.P. 2002. DNA points the way ahead in taxonomy // Nature. Vol.418. P.479.

Tautz D., Arctander P., Minelli A., Thomas R.H. & Vogler A.P. 2003. A plea for DNA taxonomy // Trends in Ecology and Evolution. Vol.18. P.70–74.

Wilson E.O. 2004. Taxonomy as a fundamental discipline // Phil. Trans. R. Soc. Lond., B. Vol.359. P.739.