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Verfasserin / Verfasser: Patrick Strutzenberger  
Matrikel-Nummer: 0200076  
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Betreuerin / Betreuer: Univ.- Prof. Dr. Konrad Fiedler

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# DNA barcoding and molecular phylogeny of *Eois* moths (Geometridae) from southern Ecuador

Patrick Strutzenberger

## Abstract

The genus *Eois* (Larentiinae) comprises an important part of a megadiverse assemblage of geometrid moths in the mountain rainforests of southern Ecuador. Owing to the diversity of morphotypes found in this genus, the abundance of many of its component species, and the close relationships with larval food plants in the genus *Piper*, the genus *Eois* was chosen as a target for more detailed investigation on the suitability of DNA barcode applications and phylogenetic relationships. COI barcode sequences (676 bp) were generated from 342 individuals to establish an inventory of DNA barcodes from one study area (Reserva Biológica San Francisco). This inventory can be used to match larvae to known adult samples without rearing, and will therefore be of vital help to extend the currently very limited knowledge about food plant relationships and host specialization. Analyses of barcoding sequence divergence showed that species delimitations based on external morphology alone result in broad overlap of intra- and interspecific distances. Species delimitation at a 3% pairwise genetic distance threshold eliminated all but 3 out of 9 cases causing overlap and delimitation at a 2% threshold resulted in a clear barcoding gap. 30 previously unrecognized species were identified solely on morphological evidence, and their species status was subsequently supported by barcode sequence data. In addition to that 9 putative species could be recognized by threshold based delimitation. Hence, the present study increased the number of *Eois* species recorded from that small area of Andean mountain forests from 99 to 129 (morphology-based) or even 138 (sequence-based). Notably there are no cases where two or more previously known morphospecies had to be lumped. We acquired elongation factor 1-alpha sequences and extended COI sequences of 102 individuals amounting to a combined sequence dataset of ~2200 bp. These represented at least 70 *Eois* species plus a range of outgroup taxa. Maximum parsimony and Bayesian inference of phylogeny were employed to estimate relationships within *Eois*. Monophyly of *Eois* was always strongly supported. Phylogenetic trees revealed that all included wing-pattern types except for one evolved only once. The exception to this rule occurred in the most basal clade whose species closely resemble those in a clade higher up in the tree. This may represent an ancestral character state or the result of convergent evolution. Additionally it could be determined that species known to feed on *Piper* are spread over most of the major clades within *Eois*, indicating that the host plant relationship with *Piper* is indeed a widespread trait among the Neotropical members of the genus and not just occurs in certain subclades. The number of species and individuals recorded from *Piper* is however skewed towards two subclades. Within the subfamily Larentiinae the genus *Eois* has traditionally been placed close to the tribe Eupitheciini, but there is presently no support for such a placement in our phylogenetic analyses.

## Deutsche Zusammenfassung

Die Gattung *Eois* (Larentiinae) ist Teil einer megadiversen Fauna von Geometriden in den Bergregenwäldern des südlichen Ecuador. Angesichts der hohen Diversität an Morphotypen und der engen Beziehung zu Futterpflanzen aus der Gattung *Piper* wurde die Gattung *Eois* zur weiterführenden Untersuchung ausgewählt. Hauptfragestellungen waren die Brauchbarkeit von Barcode-Ansätzen zur Artidentifikation sowie ein erster Einblick in phylogenetische Zusammenhänge innerhalb von *Eois*. COI-Barcode-Sequenzen von 340 Individuen wurden generiert, um eine Datenbank der Artengemeinschaft an der Sammlungslokalität zu erstellen. Dieses Inventar wird von großem Nutzen bei der Identifikation von Raupen sein und damit die Zahl der Futterpflanzennachweise ohne die

Notwendigkeit zeitraubender Zuchten erhöhen. Die Analyse der Barcoding-Sequenzen zeigte, dass es im Fall einer rein Morphologie-basierten Artabgrenzung zu einer breiten Überlappung von intra- und interspezifischen genetischen Distanzen kommt. Artabgrenzung auf Sequenzbasis mit einem Schwellenwert von 3% eliminiert 6 von 9 Fällen, die eine Überlappung verursachen. Abgrenzung mit einem 2% Schwellenwert führt zu einem klaren "barcoding gap". Es konnten allein aufgrund der Flügelzeichnungsmerkmale 30 neue Morphospezies im vorliegenden Material identifiziert werden, deren Artstatus sich ausnahmslos durch Analyse der DNA-Barcode-Sequenzen bestätigte. Die Zahl der *Eois*-Arten in diesem kleinen, nur wenige Quadratkilometer großen Areal an Bergregenwald steigt daher von 99 auf 129. Diese Zahl erhöht sich weiter (auf 138), wenn 9 potentielle Arten, welche durch genetische Artabgrenzung unter Anwendung eines 2% Schwellenwertes identifiziert wurden, mit eingerechnet werden. Erweiterte COI Sequenzen (1220bp) und *Elongation-factor-1-alpha* Sequenzen (1066bp) von 102 Taxa wurden generiert. *Maximum parsimony* und Bayes'sche Methoden der Phylogenierekonstruktion wurden angewandt, um die Verwandtschaftsverhältnisse innerhalb *Eois* abzuschätzen. Alle Analysen bestätigten die Monophylie der untersuchten neotropischen Vertreter der Gattung *Eois*. Die Stammbäume zeigen, dass alle betrachteten Flügelmuster-Typen – bis auf eine Ausnahme – nur einmal entstanden sind. Diese Ausnahme betrifft den basalen Clade innerhalb *Eois*, welcher einem abgeleiteteren Clade ähnelt. Dies könnte den plesiomorphen Zustand repräsentieren oder ein Fall konvergenter Evolution sein. Futterpflanzenbeziehungen zu *Piper* sind über die gesamte Gattung hinweg zu finden. Jedoch sind die vorliegenden Nachweise auf bestimmte Subclades konzentriert. Die bislang in der Literatur vermutete nahe phylogenetische Verwandtschaft der Gattung *Eois* mit dem Tribus Eupitheciini (Blütenspanner) konnte anhand der molekularen Daten nicht bestätigt werden.

## 1. Introduction

*Eois* is a speciose genus of rather small-sized geometrid moths and does as such comprise an important part of a megadiverse assemblage of geometrid moths in the mountain rainforests of southern Ecuador (Brehm, 2002). Over the past 10 years, the geometrid fauna of one particular locality, the Estación Científica San Francisco (ECSF), situated in the Reserva Biológica San Francisco (RBSF), has served as a paradigm to investigate patterns in species diversity and community structure of tropical moths (e.g. Brehm, 2002; Brehm et al., 2003a, 2003b and 2005; Brehm and Fiedler, 2003 and 2005; Fiedler et al., 2008). These studies form a part of a multidisciplinary research initiative funded by the German Science Foundation (<http://www.bergregenwald.de>, <http://www.tropicalmountainforest.org>). The genus *Eois* is described from the Americas, ranging from Mexico to Argentina, as well as from SE Asia, Australia and Africa (Scoble, 1999). Scoble (1999) recognized 247 species, the majority of which (209) occur in the Neotropical region. According to Brehm et al. (2005) at least 56 *Eois* species occur in the RBSF area between 1040-2700 m elevation. In the Neotropics a number of hostplant records for *Eois* could be gained in recent years as part of massive campaigns to elucidate tropical food webs. All published hostplant records for Neotropical *Eois* are from *Piper* plants (Bodner, 2007; Dyer et al., 2002 and 2008; Dyer and Palmer, 2006; Janzen and Hallwachs, 2005). This apparently close relationship with *Piper* along with the high morphological diversity observed within *Eois* render this genus a promising target for investigation of evolutionary patterns and ecological processes. Especially, the monophyly of the morphologically diverse genus, the phylogenetic distribution of affiliations with Piperaceae hostplants, and identification of possible candidates for the sister-group of *Eois* are relevant topics for a molecular phylogenetic study.

Current research on moths in the RBSF study area has a focus on caterpillar ensembles on shrubs in the montane forest zone. Larvae are collected and then reared to adulthood for identification, in order to establish biological networks between phytophagous insects and their hostplants. This approach is often troubled by high mortality of caterpillars as well as by the massive need for manpower. In this situation identification of larvae through DNA barcodes, without the need of rearing individuals to

**Table 1.** Primer sequences and PCR and sequencing reaction cycler programs for all primers used in this study.

Gene Fragment	Primer pairs	Reference	Cylcer program
5' part of COI (barcoding sequence)	LCO1490: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' Nancy: 5'-CCC GGT AAA ATT AAA ATA TA ACT TC-3'	Folmer et al. (1993)	94°C/4 min; 94°C/1 min, 44°C/1.30 min, 72°C/1.30 min, 5 cycles; 94°C/1 min, 49°C/1 min, 72°C/1 min, 35 cycles; 72°C/7 min
	LCO1490: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' HCO2198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'	Folmer et al. (1993)	same as LCO1490/Nancy
3' part of COI	Jerry: 5'-CAA CAT TTA TTT TGA TTT TTT GG-3' Hobbes: 5'-AAA TGT TGN GGR AAA AAT GTT A-3'	Simon et al. (1994)	same as LCO1490/Nancy
	Tonya: 5'-GAA GTT TAT ATT TTA ATT TTA CC GGG-3' Hobbes: 5'-AAA TGT TGN GGR AAA AAT GTT A-3'	Simon et al. (1994)	same as LCO1490/Nancy
5' part of EF-1 $\alpha$	EF44: 5'-GCY GAR CGY GAR CGT GGT ATY AC-3' Verdi4: 5'-CAC CAG TCT CCA CAC GGC-3'	Monteiro and Pierce (2001)	95°C/7 min; 95°C/1 min, 51-56.5°C/1-1.30 min, 72°C/1-1.30 min, 35-40 cycles; 72°C/10 min
	EF44: 5'-GCY GAR CGY GAR CGT GGT ATY AC-3' Verdi: 5'-GAC ACC AGT TTC IAC TCT GCC-3'	Monteiro and Pierce (2001)	same as EF44/Verdi4
	Cho2: 5'-CTA GGT CAC CAT CAT CGA-3' Verdi4: 5'-CAC CAG TCT CCA CAC GGC-3'	Snäll et al. (2007)	same as EF44/Verdi4
3' part of EF-1 $\alpha$	EF51.9: 5'-CAR GAC GTA TAC AAA ATC-3' EFrcM4: 5'-ACA GCV ACK GTY TGY CTC ATR TC-3'	Monteiro and Pierce (2001)	95°C/7 min; 95°C/1 min, 51-54°C/1-1.30 min, 72°C/1-1.30 min, 35-40 cycles; 72°C/10 min
	Niina: 5'-CCA CAG ACA AGG CCC TGC G-3' Niina2: 5'-CCT GGA AGG ACT CCA CRC ACA G-3'	Snäll et al. (2007)	95°C/7min; 95°C/1min, 53°C/1min, 72°C/1.30min, 35 cycles; 72°C/10min
Sequencing reactions	all		96°C/2 min; 96°C/20 s, 48°C/20 s, 60°C/4 min, 25 cycles; 60°C/4 min

adulthood, could provide an elegant solution. In recent years DNA barcoding has been proposed as a quick and increasingly cheap tool for species level identification of all Metazoa and eventually all eukaryotes. A 658bp fragment from the 5' part of the mitochondrial cytochrome c oxidase subunit I (COI) gene, as amplified by the primers LCO1490/HCO2198 described by Folmer et al. (1994), has been proposed as a universal marker for all Metazoa (Hebert et al., 2003a and 2003b). The conceptual foundation for DNA barcoding is the assumption that interspecific genetic distances exceed intraspecific distances by such a margin that a distinct gap exists. The presence of this so called barcoding gap allows one to set a threshold for species discrimination. Sequence pairs with distances below the threshold are thought to be conspecific, whereas pairs with distances above the threshold are accepted to belong to separate species.

Proponents of DNA barcoding claim that this assumption is true in virtually all cases and deviations are either caused by a negligible number of cases of incomplete lineage sorting or can be attributed to shortcomings in traditional taxonomy (i.e. failure to recognize cryptic species) of the taxa in question (Barrett and Hebert, 2005; Hajibabaei et al., 2006; Hebert et al., 2003a, 2003b and 2004). The universal presence of a "barcoding gap" would then allow for instant species delimitation even in previously entirely unknown taxa. However a number of case studies, where the power of DNA barcoding to distinguish morphologically or otherwise well defined species has been put under scrutiny, show that this is not always the case. Recent examples that demonstrate problems with the assumption of a barcoding gap come from as diverse arthropod groups such as lycaenid butterflies (Wiemers and Fiedler, 2007), ithomiine butterflies (Elias et al., 2007), orthopterans (Trewick, 2008), harvestmen (Boyer et al., 2007), or flies (Meier et al., 2006). However, with the possible exception of the study by Wiemers and Fiedler (2007) none of these case studies is immune to the insufficient taxonomy argument. It is quite obvious that the success of identification through barcoding is crucially dependent on comprehensive taxon sampling. The short barcoding sequences contain only very limited phylogenetic information and correct assignments by distance based methods do require quite closely related sequences to compare to. Furthermore, barcoding is bound to fail when applied to non-monophyletic species which may be present in recently diverged species and in cases of hybridization. Many publications tried to highlight the conceptual shortcomings of DNA barcoding, the reader is therefore referred to two of the most profound and unbiased ones, namely DeSalle et al. (2005) and Meyer and Paulay (2005).

The present study pursues two principal aims: (a) to establish a DNA barcode library of *Eois* moths from the study area and test its utility against species delimitations and identifications that had been obtained earlier using conventional (i.e. morphological) taxonomic methods; and (b) to explore phylogenetic patterns in the genus *Eois* by means of a combination of mitochondrial and nuclear sequence data.

## 2. Material and methods

### *Collection and DNA sequencing*

Most moths used in this study were collected from a small area of mountain ravine (quebrada) forests (approximately 1km<sup>2</sup> in extension) in the surroundings of the Estación Científica San Francisco. Collection sites for all specimens used in this study are indicated in Table 2 and A4, for details about the area see Zimmermann (2005) and Günter et al. (2008). Some representatives of possible outgroups were also collected at other sites (see Table 2). Moths were killed with cyanide, dried and stored at -20°C until extraction. All specimens used for sequence analyses had been stored for less than 4 years. Heads and/or legs of moths were homogenized with ceramic beads using a Precellys 24 homogenizer set to 5000 min<sup>-1</sup> for 2x20 s. DNA extraction was performed with the DNEasy Tissue Kit (Qiagen) or the Peqgold Tissue DNA mini Kit (Peqlab) according to the protocol supplied with the respective kit. The target fragments were amplified using the Fermentas PCR system. PCR reactions were set up with

**Table 2.** List of taxa included in phylogenetic analyses for sequences generated in this study with length of sequences indicated. Asterisk denotes chimeric taxa. Numeric species codes are given for reference to G. Brehm's database when applicable.

Taxon	Code	Collection site	COI	Efla
<b>Larentiinae</b>				
<i>Ecliptopera silaceata</i>	Eo00391	Orth a. d. Donau, Austria	1220bp	1066bp
<i>Eois angulata</i>	Eo00255	Q5#1 RBSF, Ecuador	1220bp	1066bp
<i>Eois antiopata</i>	Eo00023	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois biradiata</i>	Eo00008	Q3#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois burla</i>	Eo00080	Q2#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois camptographata</i>	Eo00345	BC-II RBSF, Ecuador	1220bp	1066bp
<i>Eois carrasca</i>	Eo00143	Q5#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois catana</i>	Eo00352	4a RBSF, Ecuador	1187bp	1066bp
<i>Eois chasca</i>	Eo00096	Q5#3 RBSF, Ecuador	676bp 5'only	1066bp
<i>Eois chyrsocraspedata</i>	Eo00077	Q2#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois ciocolatina</i>	Eo00097	Q2#3 RBSF, Ecuador	1188bp	1066bp
<i>Eois escamata</i>	Eo00035	Q2#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois inflammata</i>	Eo00060	Q3#3 RBSF, Ecuador	1220bp	1027bp
<i>Eois isographata</i>	Eo00382	Costa Rica	1170bp	1066bp
<i>Eois lucivittata</i> 384	Eo00283	Q3#3 RBSF, Ecuador	1220bp	588bp 5' only
<i>Eois lunifera</i>	Eo00042	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois multistrigaria</i>	Eo00386	Costa Rica	1220bp	1066bp
<i>Eois muscosa</i>	Eo00045	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois necula</i>	Eo00125	Q3#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois nigrosticta</i> 388	Eo00340	BC-I RBSF, Ecuador	1220bp	1066bp
<i>Eois olivaria</i>	Eo00090	Q2#1 RBSF, Ecuador	1220bp	1066bp
<i>Eois pallidicosta</i>	Eo00244	Q5#3 RBSF, Ecuador	1220bp	1066bp*
<i>Eois plana</i>	Eo00040	Q3#1 RBSF, Ecuador	1220bp	1039bp
<i>Eois planetaria</i>	Eo00150	Q3#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois sagittaria</i>	Eo00166	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp	Eo00226	Q3#1 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp 1070	Eo00103	Q5#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp 2128	Eo00323	Q3#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp 405	Eo00173	Q3#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp 409	Eo00063	Q2#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp 420	Eo00205	Q3#1 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp 427	Eo00019	Q3#1 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp 820	Eo00048	Q3#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp.	Eo00082	Q3#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp.	Eo00290	Q3#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp.	Eo00339	BC-IV RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp.	Eo00349	BC-VI RBSF, Ecuador	1208bp	1066bp
<i>Eois</i> sp.	Eo00065	Q2#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> sp.	Eo00331	Q5#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> spnr 2129	Eo00326	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> spnr 2133	Eo00095	Q5#3 RBSF, Ecuador	1220bp	985bp
<i>Eois</i> spnr <i>adimaria</i> 399	Eo00188	Q5#3 RBSF, Ecuador	1193bp	1066bp
<i>Eois</i> spnr <i>azafranata</i>	Eo00154	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> spnr <i>azafranata</i>	Eo00281	Q3#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> spnr <i>basaliata</i>	Eo00245	Q5#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> spnr <i>biradiata</i>	Eo00227	Q3#1 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> spnr <i>cancellata</i>	Eo00003	Q2#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> spnr <i>catana</i>	Eo00013	Q3#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> spnr <i>chasca</i>	Eo00107	Q5#3 RBSF, Ecuador	1220bp	586bp 5' only
<i>Eois</i> spnr <i>ciocolatina</i>	Eo00034	Q2#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> spnr <i>ciocolatina</i>	Eo00381	4a RBSF, Ecuador	1196bp	1066bp
<i>Eois</i> spnr <i>concatenata</i>	Eo00238	Q5#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> spnr <i>dorisaria</i>	Eo00190	Q2#1 RBSF, Ecuador	1220bp	1066bp
<i>Eois</i> spnr <i>encina</i>	Eo00149	Q3#2 RBSF, Ecuador	1220bp	1066bp

<i>Eois spnr fucosa</i>	Eo00163	Q3#1 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr golosata</i>	Eo00012	Q3#3 RBSF, Ecuador	1210bp	1066bp
<i>Eois spnr golosata</i>	Eo00028	Q3#2 RBSF, Ecuador	1220bp	559bp 5' only
<i>Eois spnr golosata</i>	Eo00219	Q3#2 RBSF, Ecuador	1220bp	585bp 5' only
<i>Eois spnr golosata</i>	Eo00307	Q5#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr goodmani</i>	Eo00014	Q3#3 RBSF, Ecuador	1220bp	1013bp
<i>Eois spnr goodmani</i>	Eo00044	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr goodmani</i>	Eo00133	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr goodmani</i>	Eo00144	Q3#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr goodmani</i>	Eo00231	Q5#1 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr haematodes 977</i>	Eo00187	Q5#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr inflammata 515</i>	Eo00098	Q2#3 RBSF, Ecuador	1220bp	1066bp*
<i>Eois spnr margarita</i>	Eo00010	Q3#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr margarita</i>	Eo00002	Q3#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr nigrostricta</i>	Eo00218	Q5#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr nigrostricta 388</i>	Eo00180	Q2#3 RBSF, Ecuador	1220bp	1004bp
<i>Eois spnr odati 419</i>	Eo00073	Q3#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr olivacea</i>	Eo00031	Q2#1 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr olivacea</i>	Eo00085	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr olivacea</i>	Eo00087	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr olivacea</i>	Eo00263	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr olivacea</i>	Eo00335	BC-IV RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr pallidicosta</i>	Eo00217	Q5#3 RBSF, Ecuador	1220bp	526bp 3' only
<i>Eois spnr pallidicosta</i>	Eo00239	Q5#2 RBSF, Ecuador	1220bp	527bp 3' only
<i>Eois spnr paraviolascens</i>	Eo00024	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr paulona</i>	Eo00353	4a RBSF, Ecuador	1220bp	522bp 3' only
<i>Eois spnr sagittaria</i>	Eo00214	Q3#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr sp. 2133</i>	Eo00041	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr tegularia 961</i>	Eo00099	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr tegularia 961</i>	Eo00139	Q2#3 RBSF, Ecuador	1220bp	1035bp
<i>Eois spnr trillista 441</i>	Eo00111	Q2#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr xanthoperata</i>	Eo00066	Q2#2 RBSF, Ecuador	1220bp	1066bp*
<i>Eois spnr yvatta 400</i>	Eo00350	BC-VI RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr. borrata</i>	Eo00181	Q2#3 RBSF, Ecuador	1178bp	1066bp
<i>Eois spnr. borrata</i>	Eo00282	Q3#3 RBSF, Ecuador	1220bp	1066bp
<i>Eois spnr. borrata</i>	Eo00333	Q5#2 RBSF, Ecuador	1220bp	1066bp
<i>Eois tegularia 836</i>	Eo00016	Q3#1 RBSF, Ecuador	1220bp	1066bp
<i>Epirrhoe alternata</i>	Eo00396	Orth a. d. Donau, Austria	1220bp	1066bp
<i>Eulithis pyraliata</i>	Eo00398	Orth a. d. Donau, Austria	1220bp	1066bp
<i>Eupithecia impurata</i>	Eo00405	Gesäuse NP, Austria	1220bp	1066bp
<i>Eupithecia linariata</i>	Eo00402	Gesäuse NP, Austria	1220bp	1066bp
<i>Pasiphila rectangulata</i>	Eo00401	Orth a. d. Donau, Austria	1220bp	1028bp
<i>Perizoma minoratum</i>	Eo00407	Gesäuse NP, Austria	1220bp	1066bp
<i>Philereme transversata</i>	Eo00393	Orth a. d. Donau, Austria	1220bp	1066bp
<i>Xanthorhoe quadrifasciata</i>	Eo00395	Orth a. d. Donau, Austria	1220bp	913bp
<b>Sterrhinae</b>				
<i>Idaea quadrirubata</i>	Eo00211	Q3#3 RBSF, Ecuador	1220bp	513bp 3' only
<i>Idaea spnr quadrirubata</i>	Eo00347	BC-II RBSF, Ecuador	1220bp	526bp 3' only
<i>Lobocleta costalis</i>	Eo00344	BC-II RBSF, Ecuador	676bp 5' only	523bp 3' only

2.5 µl of 10x (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> PCR Buffer, 2 µl 25mM MgCl<sub>2</sub>, 0.1 µl 10mM dNTPs, 1 µl of each primer, 1µl genomic DNA, 1 µl Taq Polymerase and filled to 25µl with PCR grade H<sub>2</sub>O. PCR reactions were purified by digestion with Shrimp Alkaline Phosphatase and Exonuclease for 15 min at 37°C followed by 15min at 80°C for enzyme deactivation. Sequencing reactions were set up with 1 µl ABI BigDye 3.1, 1µl primer, 1 µl template DNA and filled to 10 µl with PCR grade H<sub>2</sub>O and sequenced on an ABI capillary sequencer. All gene fragments were sequenced in both directions. PCR and sequencing primers as well as thermal cycler programs are indicated in Table 1.



**Table 3.** Outgroup sequences from Genbank included in phylogenetic analyses with their genbank accession numbers and length indicated.

Taxon	COI	Length	Reference	Efl $\alpha$	Length	Reference
<b>Archiearinae</b>						
<i>Archiearis parthenias</i>	DQ018928	1450bp	Wahlberg (2005)	DQ018899	1225bp	Wahlberg (2005)
<b>Larentiinae</b>						
<i>Anticollix sparsata</i>	EU443353	1536bp	Ounap (2008)	EU443291	883bp	Ounap (2008)
<i>Epirrhone hastulata</i>	EU443351	1536bp	Ounap (2008)	EU443289	883bp	Ounap (2008)
<i>Hydriomena impluviata</i>	EU443350	1536bp	Ounap (2008)	EU443288	883bp	Ounap (2008)
<i>Lythria cruentaria</i>	EU443365	1536bp	Ounap (2008)	EU443302	883bp	Ounap (2008)
<i>Lythria purpuraria</i>	EU443367	1536bp	Ounap (2008)	EU443304	883bp	Ounap (2008)
<i>Lythria sanguinaria</i>	EU443366	1536bp	Ounap (2008)	EU443303	883bp	Ounap (2008)
<i>Phibalapteryx virgata</i>	EU443352	1536bp	Ounap (2008)	EU443290	883bp	Ounap (2008)
<i>Trichopteryx carpinata</i>	EU443349	1536bp	Ounap (2008)	EU443287	883bp	Ounap (2008)
<b>Sterrhinae</b>						
<i>Cyclophora albipunctata</i>	EU443360	1536bp	Ounap (2008)	EU443297	883bp	Ounap (2008)
<i>Cyclophora punctaria</i>	EU443361	1536bp	Ounap (2008)	EU443298	883bp	Ounap (2008)
<i>Haematopsis grataria</i>	EU443364	1536bp	Ounap (2008)	EU443301	883bp	Ounap (2008)
<i>Idaea aversata</i>	EU443357	1536bp	Ounap (2008)	EU443294	883bp	Ounap (2008)
<i>Idaea straminata</i>	EU443356	1536bp	Ounap (2008)	AY948507	904bp	Snäll (2007)
<i>Rhodometra saccharia</i>	AJ870398	1536bp	Knoelke (2004)	EU443305	883bp	Ounap (2008)
<i>Rhodostrophia calabra</i>	EU443355	1536bp	Ounap (2008)	EU443293	883bp	Ounap (2008)
<i>Rhodostrophia vibicaria</i>	EU443354	1536bp	Ounap (2008)	EU443292	883bp	Ounap (2008)
<i>Scopula decorata</i>	EU443359	1536bp	Ounap (2008)	EU443296	883bp	Ounap (2008)
<i>Timandra comae</i>	EU443363	1533bp	Ounap (2008)	EU443300	883bp	Ounap (2008)
<i>Timandra griseata</i>	EU443362	1533bp	Ounap (2008)	EU443299	883bp	Ounap (2008)

#### *Morphological examination of moths*

Wings of the right body side were cut off and glued to a piece of cardboard. Photographs in dorsal and ventral view were taken on an Olympus optical device (natural size), processed and examined using Adobe Photoshop versions 8 and 9 and Cell\_D version 3.2. The state of taxonomy of *Eois* is poor, and many species recognized by morphological sorting from Ecuador are still formally undescribed. Identity of species is often unclear and examination of further type specimens preserved in museum collections will be necessary in many cases. The samples gathered at the ECSF in the past years had been determined on morphospecies level only, using extensive collections in the natural history museums of London, Munich, and New York as templates (Brehm et al., 2005). Moths used for sequence analyses were first determined on morphospecies level by the author as well as Konrad Fiedler and Gunnar Brehm using an image database of Ecuadorian geometrids that had been compiled by Gunnar Brehm in the course of the aforementioned biodiversity studies. All *Eois* morphotypes that could not be matched to morphospecies already contained in the image database were treated as separate entities. In light of these caveats, most taxonomic assignments in this work are to be taken as preliminary.

#### *Sequence data processing*

Proofreading of sequences and contig assembly was done with ChromasLite Version 2.01, ChromasPro Ver 1.34 and DNASTar Lasergene SeqMan Pro Ver. 7.1. Assembled sequences have a length of 1220 bp and 1066 bp for COI and Efl $\alpha$ , respectively. All sequences were aligned manually using Bioedit Ver 7.0.4.1 (Hall, 1999). Sequence data was prepared for analyses using the programs FORCON version 1.0 and MEGA versions 3 and 4 (Kumar et al., 2004; Tamura et al., 2007). Sequences were

screened for unusual nucleotide composition and the presence of erratic stop codons to control for possible pseudogene amplification.

#### *DNA barcoding analyses*

Pairwise Kimura-2-parameter distances (Kimura, 1980) were calculated from the 676bp barcoding fragment of the COI gene with PAUP\*. This particular measure of genetic distance has been chosen to facilitate comparability with other DNA barcoding studies where it has been used extensively. Distances were analyzed with Microsoft Excel Version 10. Neighbor joining trees were calculated and visualized using MEGA versions 3 and 4. For neighbor joining analyses we supplemented the sequence dataset with outgroup sequences from Genbank as indicated in Table A6. In the analysis of barcoding sequences a comparison has been made between species delimited by a sequence divergence threshold of 3% and 2%, respectively, and species delimited on morphospecies level. Morphospecies were defined after morphological examination (see above), and through the application of an integrative taxonomy approach in cases where the amount of sequence divergence between seemingly conspecific individuals made the recognition of morphological differences possible in hindsight. Threshold based species were defined using neighbor joining phylograms. Every monophyletic clade with at least one internal sequence pair with a distance of less than the threshold value was considered one species. Species represented by only a single individual (36 species altogether) were excluded from analyses of intra- versus interspecific genetic distances.

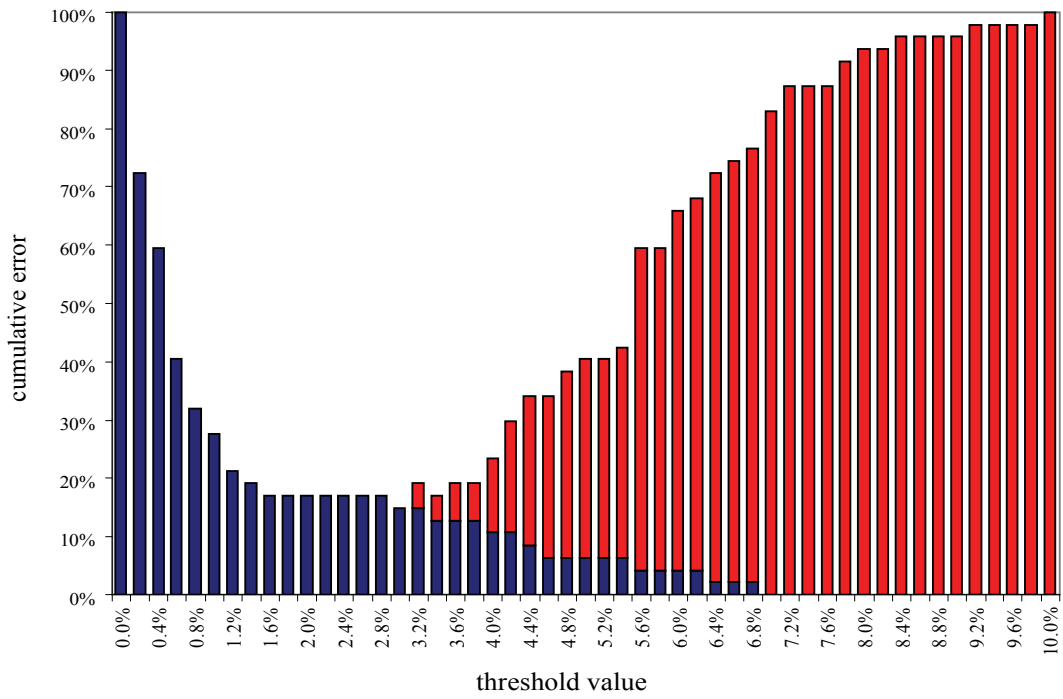
#### *Sequence dataset for phylogenetic analysis*

Proofreading of sequences and contig assembly was done with ChromasLite Version 2.01, ChromasPro Ver 1.34 and DNASTar Lasergene SeqMan Pro Ver. 7.1. Assembled full length sequences have a length of 1220 bp and 1066 bp for COI and Efl $\alpha$  respectively. All sequences were aligned manually using Bioedit Ver 7.0.4.1 (Hall, 1999). Sequence data was prepared for analyses using the programs FORCON version 1.0 and MEGA versions 3 and 4 (Kumar et al., 2004; Tamura et al., 2007). In three instances where amplification of any part of the Efl $\alpha$  gene was unsuccessful chimeric taxa were assembled using Efl $\alpha$  gene sequences gained from closely related conspecific individuals as determined by COI gene sequences. Sequences for 20 taxa were added from Genbank as listed in Table 3 with their GenBank accession numbers as well as their length indicated.

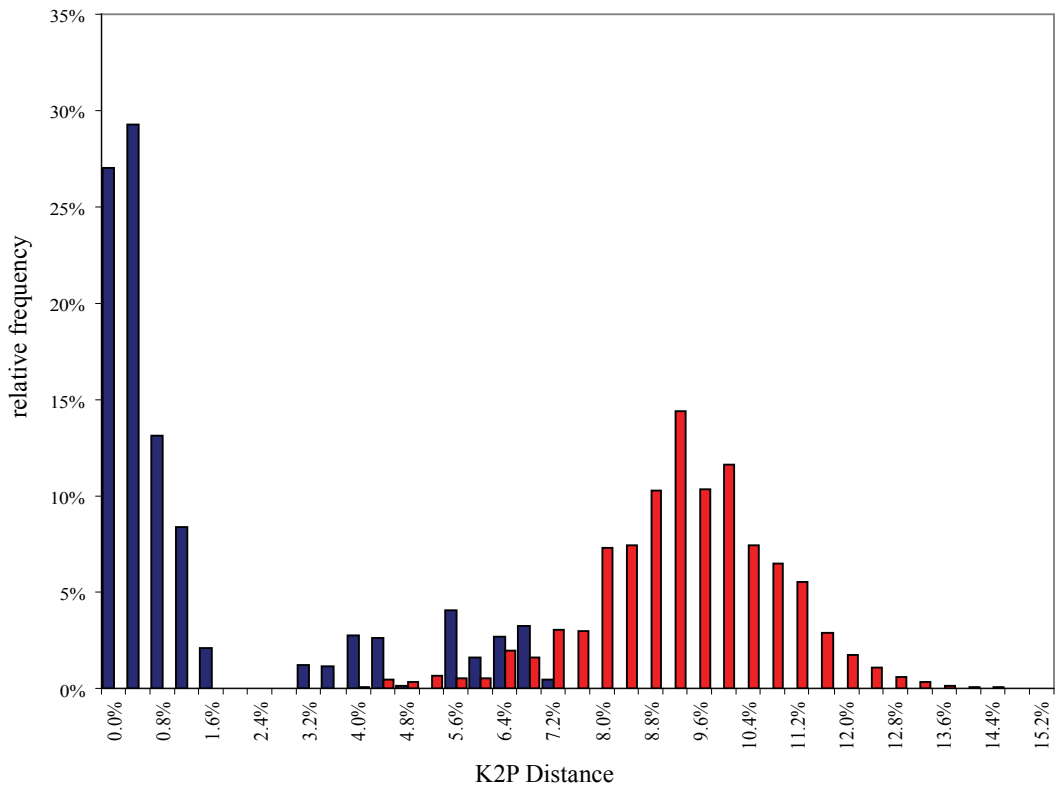
#### *Phylogenetic analyses*

Transition/transversion ratios, pairwise distances and nucleotide frequencies were calculated using PAUP\* 4b10 (Swofford, 1999) and imported into Microsoft Excel version 10 for graphic display. Maximum parsimony analyses of the combined dataset as well as for the Efl $\alpha$  gene were performed with PAUP\* 4b10 set to 100 replicates of heuristic search with tree-bisection-and-reconnection branch swapping and random addition sequence. For the COI gene the heuristic search was set to 1000 replicates to ensure sufficient sampling of the tree space. In the case of separate analyses of Efl $\alpha$  sequences a time limit of 100 seconds was imposed for each of the 100 replicates to prevent excessive and nonsensical swapping of nodes which can not be resolved by Efl $\alpha$  sequences. Bootstrapping (Felsenstein, 1985) was done with PAUP\* 4b10 using the bootstrap command set to 1000 replicates of 10 heuristic search replicates each, heuristic search parameters were tree-bisection-and-reconnection branch swapping and random addition sequence. For bootstrapping of Efl $\alpha$  trees a time limit of 90 seconds per heuristic search replicate was imposed for reasons stated above. Uninformative characters were excluded from all parsimony analyses. Bremer support values (Bremer, 1988) were calculated

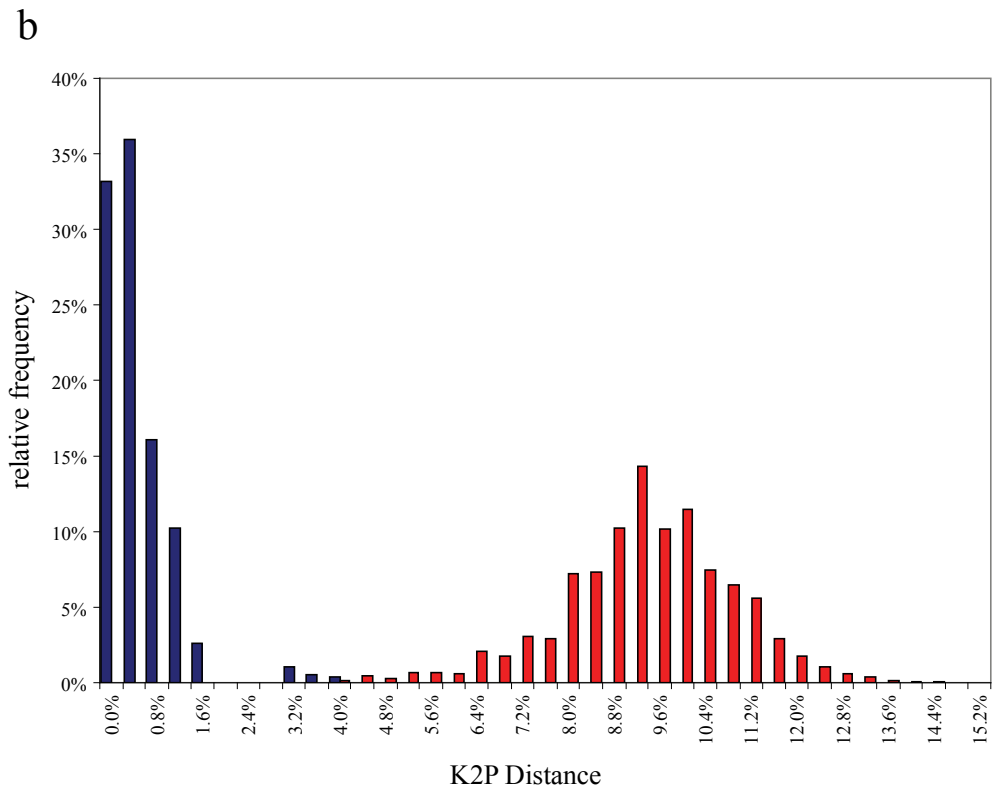
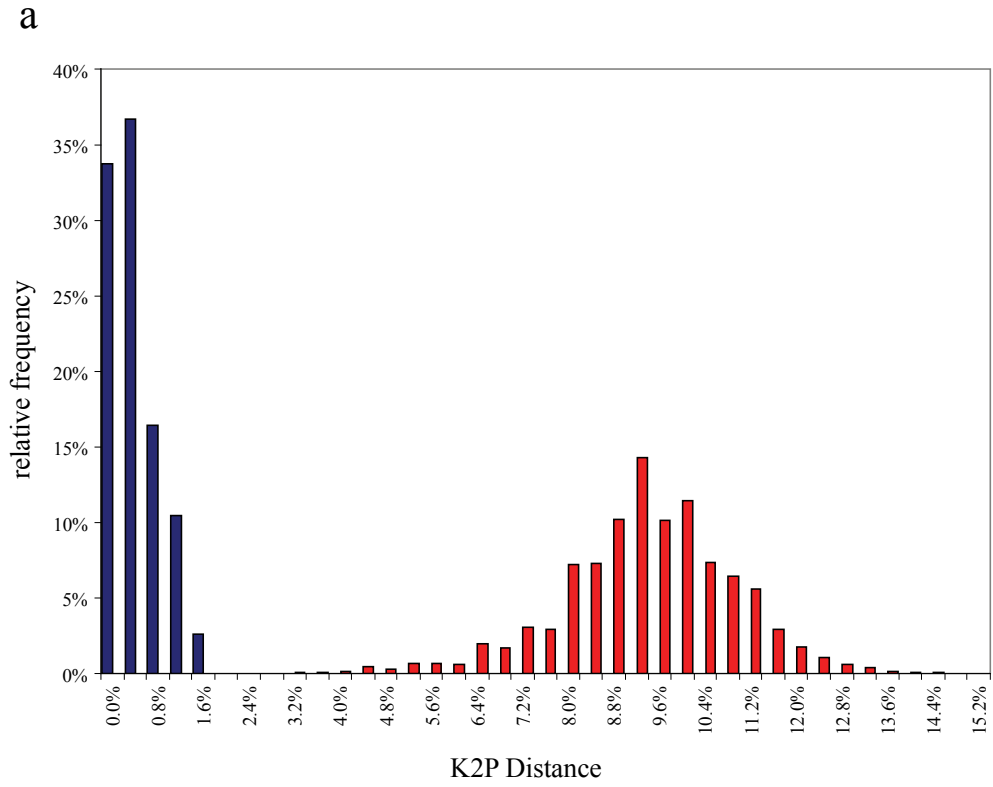
a



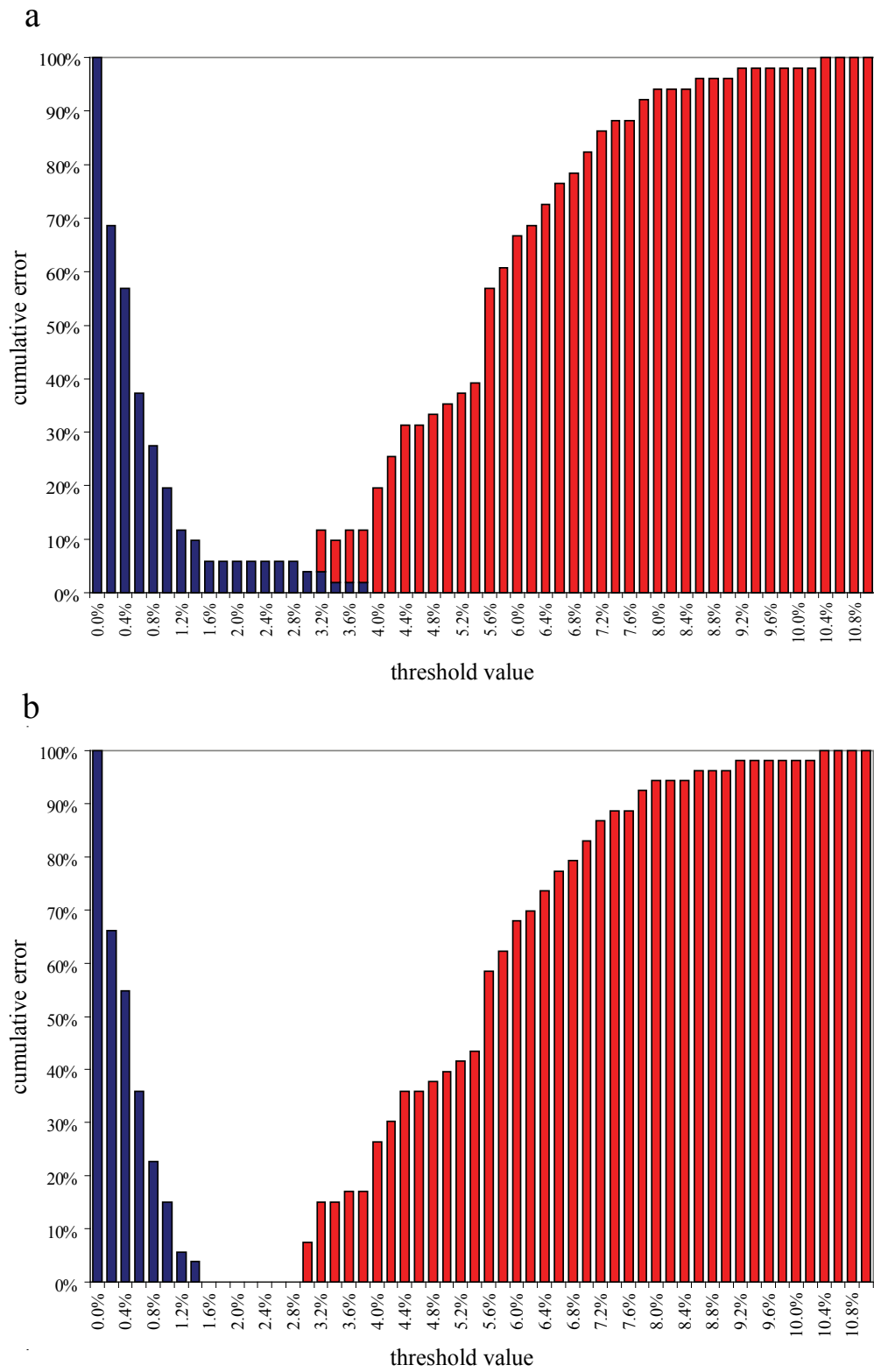
b



**Figure 1.** a: Cumulative error for given threshold values based on morphological delimitation, blue: false positives, red: false negatives; b: Plot of intra- (blue) versus interspecific (red) distances for morphological delimitation.



**Figure 2.** Plots of intra- versus interspecific pairwise distance for 2% threshold delimitation (a) and 3% threshold delimitation (b). Blue: Intraspecific distances; Red: Interspecific distances



**Figure 3.** Cumulative error rates for given threshold values for 3% threshold delimitation (a) and 2% threshold delimitation (b). Blue: false positives, red: false negatives.

using PAUP\* 4b10 in conjunction with TreeRot version 3 (Sorenson and Franzosa, 2007). Search parameters were random addition sequence and tree-bisection-reconnection branch swapping. The heuristic search was set to 100 replicates for the combined dataset and the Efl $\alpha$  gene and 500 replicates for the COI gene. Additionally a time limit of 60 seconds per replicate has been imposed for the Efl $\alpha$  gene. Strict consensus trees were calculated from the obtained most parsimonious trees using PAUP\* 4b10, bootstrap and Bremer support values were manually added to the corresponding nodes. The partition homogeneity test was performed using the hompart command implemented in PAUP with 100 heuristic search replicates, TBR branch swapping and random addition sequence. Bayesian inference of phylogeny was done with MrBayes 3.1 (Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003), the GTR+I+G model of evolution has been used as recommended by MrModeltest version 2.2 (Posada and Crandall, 1998; Nylander, 2004). For the combined analysis both genes were treated as separate partitions with the unlink option in effect.  $5 \times 10^6$  generations were used for combined analyses and  $3 \times 10^6$  generations for separate analyses of COI and EF1 $\alpha$  genes. Sample frequency was 100 in all cases. The burnin was determined by visual examination of a plot of  $-\ln L$  against the number of generations and was set to 5000, corresponding to the first 500000 generations, for all analyses. Trees were examined and edited with MEGA versions 3 and 4, Treeview version 1.6.6 (Page, 1996), Figtree version 1.1.2 and Adobe Illustrator versions 12 and 13. All trees were rooted with *Archiearis parthenias*, which has been found to lie outside of the clade (Larentiinae + Sterrhinae) in molecular as well as morphological analyses (Young, 2008; Yamamoto and Sota, 2007; Abraham et al., 2001) and is therefore well suited as outgroup in the present analysis. The inclusion of more distant outgroups produced erratic placements due to lack of phylogenetic signal.

#### *Divergence time estimation*

We applied a partitioned Bayesian dating approach (Thorne and Kishino, 2002) to trees gained from Bayesian analyses of the combined dataset as described above. Likelihood parameters were estimated using the baseml function implemented in the Paml4 package (Yang, 1997). Branch lengths were calculated with the Estbranches software (Kishino et al., 2001; Thorne and Kishino, 2002). Multidivtime was used to estimate divergence times with  $5 \times 10^6$  generations, a sampling frequency of 100 and the burnin set to 500000 generations. Fossil calibration points for Geometridae are rare, the only suitable fossil is a larentiine moth from the Florissant formation described by Cockerell (1922). A recent  $^{40}\text{Ar}/^{39}\text{Ar}$  dating of the Florissant formation (Evanoff et al., 2001) found the youngest rocks to be  $34.01\text{Ma} \pm 0.13\text{Ma}$  of age. Therefore we constrained the minimum age of Larentiinae at 34Ma.

**Table 4.** Characteristic values are given for all three means of species delimitation.

	<b>Morphology</b>	<b>3% Threshold</b>	<b>2% Threshold</b>
<b>Average Intraspecific distance</b>	1.29%	0.37%	0.32%
<b>Standard Error</b>	0.12%	0.03%	0.02%
<b>Range of Intraspecific distance</b>	0 - 6.9%	0 - 3.9%	0 - 1.5%
<b>Number of intraspecific pairs</b>	1615	1316	1290
<b>Average Interspecific distance</b>	9.15%	9.13%	9.12%
<b>Standard Error</b>	0.09%	0.09%	0.09%
<b>Range of Interspecific distance</b>	3.2 - 14.6%	3.2 - 14.6%	2.9 - 14.6%
<b>Number of interspecific pairs</b>	44441	44135	43860
<b>Number of taxa</b>	304	302	301
<b>Number of species</b>	47	51	53

### 3. Results

#### *DNA barcoding and taxonomy*

The barcoding sequence dataset comprised 360 sequences, among them 342 from *Eois*, ranging in length from 635bp to 676bp; 92.3% of sequences were of full length and the average length was 673bp. Sequence alignment was straightforward and without gaps, the alignment had a length of 676bp. No cases of NUMT amplification could be detected. Taxonomic examination of the moths by external wing characters revealed that from a total of 80 *Eois* morphospecies in the sample, 30 (~38%) were previously unknown, i.e. they could not be matched with confidence to any of the 167 morphotypes from Ecuador and Costa Rica contained in the image database. From those 30 'new' morphospecies all except for 3 are closely related, and therefore morphologically similar, to previously known morphospecies. The recognition of these morphospecies was in many cases post hoc assisted by neighbor joining diagrams calculated from barcoding sequences. Only a small number of species in our sample (24 species) could be assigned to formally described species with sufficient certainty. We screened our sample for additional, cryptic, species by application of a sequence divergence threshold; these cases are along with their COI and Efl $\alpha$  divergence values listed in Table 5. Delimitation with a 3% divergence threshold produced 6 putative additional species. Three more 'new' species could be distinguished when applying a 2% threshold value. All of these latter cases are missed by the 3% threshold only by a small margin. Note that in case of *E. spnr. azafranata* Sp042 and *E. spnr. biradiata* Sp008 the maximum interspecific distance to the sister clade is above the 3% threshold. Species are listed with new species indicated in Tables A1 (morphological delimitation) and A2 (2% and 3% threshold delimitation), respectively.

Average intra- and interspecific distances are summarized in Table 4 for morphological species delimitation as well as for delimitation with a 3% and 2% threshold. A plot of intraspecific and interspecific distances (Fig. 1b) reveals that in case of purely morphology-based delimitation there is substantial overlap of intraspecific and interspecific divergences. Analysis of cumulative error rates (Fig. 1a) shows that error is minimized with 18% at a threshold of 3% (all false positives). False positives are completely eliminated at a threshold of 7% producing 87.2% false negatives. Neighbor joining diagrams of COI barcode sequences revealed two cases of non monophyletic species when morphological delimitation is applied. When a 3% threshold for species delimitation is applied there is also some overlap between intra- and interspecific distances (Fig. 2b). Error is minimized with 4% at 3.0% threshold, all being false positives (Fig. 3a). Two of the three instances causing overlap between intra- and interspecific divergence are cases where some interspecific comparisons between the two clades in question give distances below the threshold, while others are above the threshold. False positives are completely eliminated at a threshold of 4%, producing 20% false negatives. Intraspecific distances show a pronounced bimodal distribution when morphological or 3% threshold delimitation is applied. Delimitation at 2% results in a distinct gap between 1.6% and 2.8% (Figs 2a and 3b). All specimens for which barcoding sequences were generated and used for taxonomic examination and barcoding analysis are listed in Tables A3-A5.

#### *Phylogenetic analyses*

We were able to acquire COI and Efl $\alpha$  sequences for 102 taxa. In 11 cases either COI or Efl $\alpha$  sequences were incomplete. Sequenced specimens are listed in Table 2. The sequence alignment of the COI gene has a length of 1536bp and the one for the Efl $\alpha$  gene sequences 1225bp, amounting to a combined dataset of 2761bp. Alignment was straightforward in all cases, no insertions or deletions could be detected. There are 540 parsimony informative characters in the COI gene and 345 in the Efl $\alpha$  gene, amounting to 885 informative characters for combined analyses. To assess the potential for

mutational saturation transition/transversion ratios were plotted against pairwise distances for first and second positions and separately for third codon positions (Fig. 4). It is evident that the third positions of the COI gene soon become saturated with increasing pairwise distances. No saturation was observed in the Efl $\alpha$  gene. COI sequences are AT-rich with an average AT content of 71.2% whereas Efl $\alpha$  sequences show a more balanced nucleotide composition typical for nuclear genes with an average AT content of 47.9%.

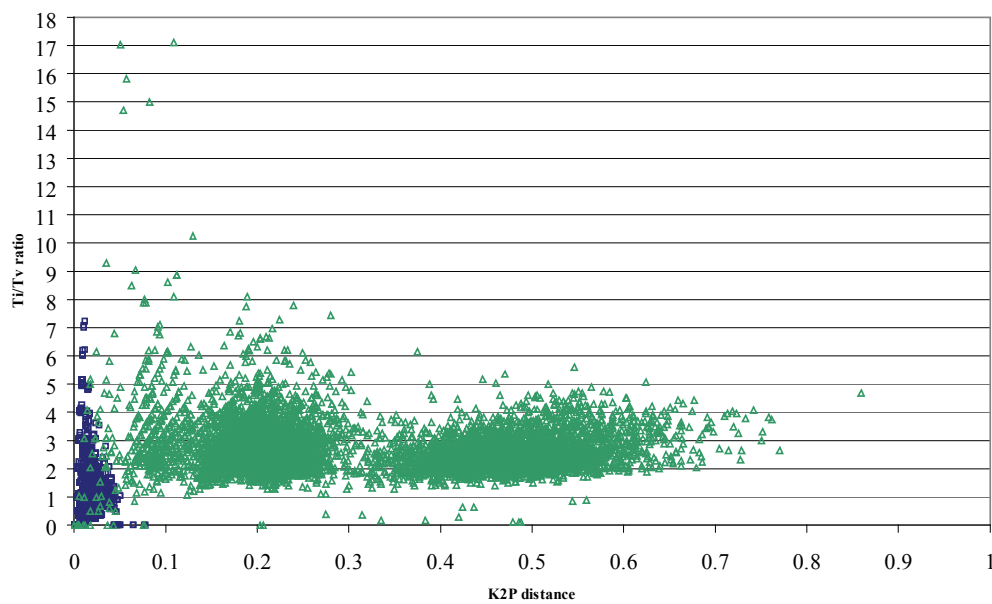
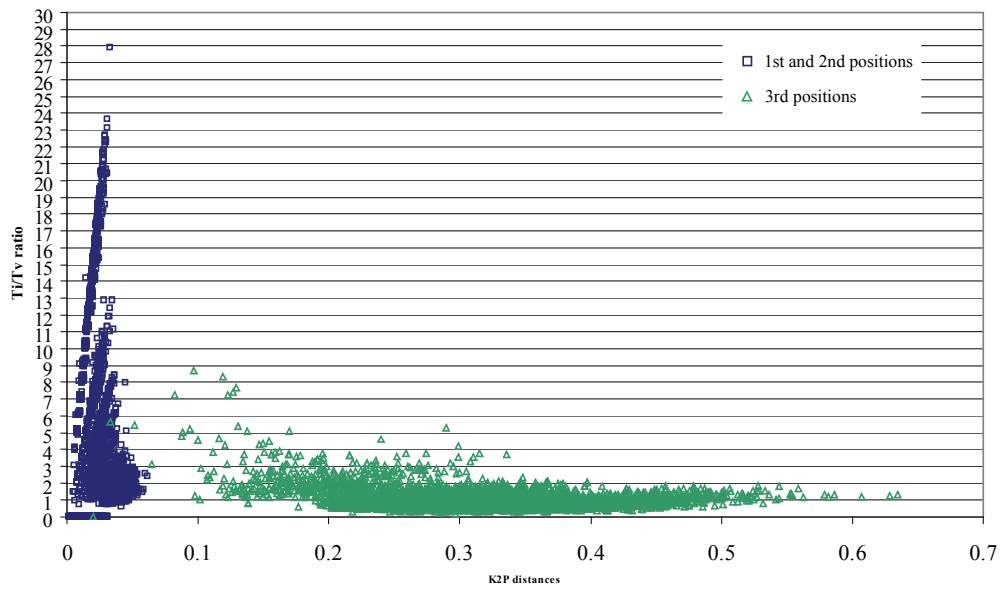
Combined maximum parsimony analyses of both genes resulted in 3 most parsimonious trees (MPTs) with a length of 9205 steps (CI=0.164, RI=0.510) (Fig 5). Analyses of the COI gene resulted in 22 MPTs with a length of 6402 steps (CI=0.142, RI=0.402) (Fig 7) and separate analysis of Efl $\alpha$  resulted in 97784 MPTs with a length of 2657 steps (CI=0.225, RI=0.685) (Fig 6). The partition homogeneity test implemented in PAUP found the resulting tree topologies to be significantly different when the two genes are compared to each other ( $p < 0.001$ ). Combined Bayesian analyses resulted in a 50% majority rule consensus tree with an average likelihood of -43863.973 (Fig 8). Single gene Bayesian analyses resulted in consensus trees (not shown) with an average likelihood of -14782.601 and -28841.435 for Efl $\alpha$  and COI, respectively. Trees calculated from both genes and Efl $\alpha$  alone are well resolved, whereas trees from single gene analyses of the COI gene are only poorly resolved.

**Table 5.** Sequence divergence values for putative species discovered by 3% and 2% threshold delimitation.

	COI min	COI max	Efl $\alpha$
<b>3% threshold</b>			
<i>E. spnr. goodmani</i> Eo00169	6.19%	6.36%	no data
<i>E. spnr. olivacea</i> Sp032	5.72%	6.87%	0.85%
<i>E. sp. Sp035</i>	5.24%	5.40%	0.85%
<i>E. spnr. golosata</i> Sp050	3.19%	3.93%	0.38%
<i>E. spnr. golosata</i> Eo00219	3.98%	4.30%	0.86%
<i>E. spnr. margarita</i> Sp052	3.94%	4.50%	0.57%
<b>2% threshold</b>			
<i>E. spnr. azafranata</i> Sp042	2.96%	3.91%	0.66%
<i>E. spnr. biradiata</i> Sp008	2.88%	3.35%	0.09%
<i>E. spnr. nigrosticta</i> 388 Eo00218	2.88%	2.88%	0.60%

*Eois* is recovered as monophyletic in the combined parsimony as well as Bayesian trees, with very high support in both cases. The same is true for analyses of the Efl $\alpha$  gene alone, whereas trees based solely on COI gene sequences fail to recover a monophyletic *Eois*. The first split within *Eois* occurs between the clade (*Eois* spnr. *catana* + *Eois catana*) and all other *Eois*. Within *Eois* 8 subclades can be distinguished whose members share certain characteristics in wing patterns and/or coloration except for one clade whose members exhibit entirely heterogeneous morphology (Fig 5). All of these clades are recovered as monophyletic in the parsimony as well as the Bayesian tree of the combined dataset and the Efl $\alpha$  gene. Only one clade, the 'yellow wing margin group', is recovered with reasonable support in single gene analyses of the COI gene. The clades are summarized in Table 6 along with a node-based definition in the sense of the PhyloCode (Cantino and deQueiroz, 2007) and their support values. Node-based definitions follow the syntax <A+B as recommended in the PhyloCode designating the least inclusive monophyletic clade including taxa A and B and are given for the purpose of clarity only. Their characteristics are described below.





**Figure 4.** Plots of transition/transversion ratio against pairwise distance for the COI gene (a) and the Efl $\alpha$  gene (b).

- Yellow with black pattern clade: Members of this clade all have yellow to orange ground coloration with black, brown, grey and sometimes iridescent spots in various patterns ranging from distinct dots to full lines. Wing shapes are uniform across the clade with rounded hindwings. The clade's internal topology differs substantially between parsimony and Bayesian analyses, being only poorly resolved in the parsimony tree.
- Inflammata species group: Named after the enigmatic *Eois inflammata* which exhibits bright pink coloration. The members of this group show in most cases a large area of their wing to be dark colored, ranging from pink over purple to dark brown. Wing shapes are uniform with rounded hind

wings. Some members also exhibit dot or line patterns reminiscent of those found in the "yellow with black pattern" group.

- Pallidicosta species group: Named after its member species *E. pallidicosta*. The members of this clade have in common a dark brown ground coloration. Most members are patterned with dark lines or patches. Definition of this clade is not straightforward as *E. planetaria* which is recovered at the base has a wildly deviating pattern and its placement is not very well supported in parsimony analyses. *E. planetaria* is however consistently associated with this clade, therefore *E. planetaria* is defined as the basalmost member of this clade.
- Yellow wing margin group: Members of this clade all exhibit a brown coloration with a yellow wing margin which can vary in intensity. Wing shapes vary from round to highly pointed wings. This clade is well supported in all analyses.
- Green/blue group: The members of this clade are colored in various shades of green and blue sometimes interrupted with fine lines. Most members also exhibit yellow wing margins as in the "yellow wing margin" group. Wing shapes range from round to pointed.
- Trillista group: A morphologically heterogeneous group, some members have a unique curved shape of the forewing. The clade has excellent support in Bayesian as well as maximum parsimony trees.
- Reticulate group: Members of this group show either some variation of a reticulate pattern or a pattern of seemingly concentric irregular shapes. This group is fully supported in the Bayesian tree and has moderately good support in maximum parsimony trees. Wings are in most cases slightly pointed.
- Cryptic species group: The cryptic species group splits into two distinct clades, the first one being defined as <*Eois tegularia* 836 & *Eois multistrigaria* is characterized by a generally lighter coloration compared to the other clade <*Eois nigrosticta* 388 & *Eois ciocolatina*. Members of this group are in general characterized by an array of cryptic patterns in light to dark brown and grey tones. Most members have a round wing shape. The clade as a whole as well as its two subclades are well supported in Bayesian as well as parsimony trees.

Relationships of these clades relative to each other are poorly supported in MP analyses, but in many cases well supported in Bayesian analyses. The most stable association is the clade <*Eois goodmani* & *Eois golosata* which has full support in Bayesian analyses and good support from MP analyses. Placements of the cryptic species group, *trillista* group and reticulate group differ between MP and Bayesian trees and are not well supported. The sister group of *Eois* in both Bayesian and MP combined analyses is a clade consisting of all other Larentiinae with only poor bootstrap support, but very strong support from Bayesian posterior probabilities. The Bayesian analysis of the *Ef1 $\alpha$*  gene however recovers *Philereme transversata* as the sister group of *Eois* with very poor support; whereas the maximum parsimony tree of the *Ef1 $\alpha$*  gene indicates *Pasiphila rectangulata* as the sister group of *Eois* with a bootstrap support value below 50. The Larentiinae are recovered as monophyletic in all trees resulting from combined analyses or analyses of the *Ef1 $\alpha$*  gene, relationships within the Larentiinae are only poorly resolved.

### *Divergence time estimation*

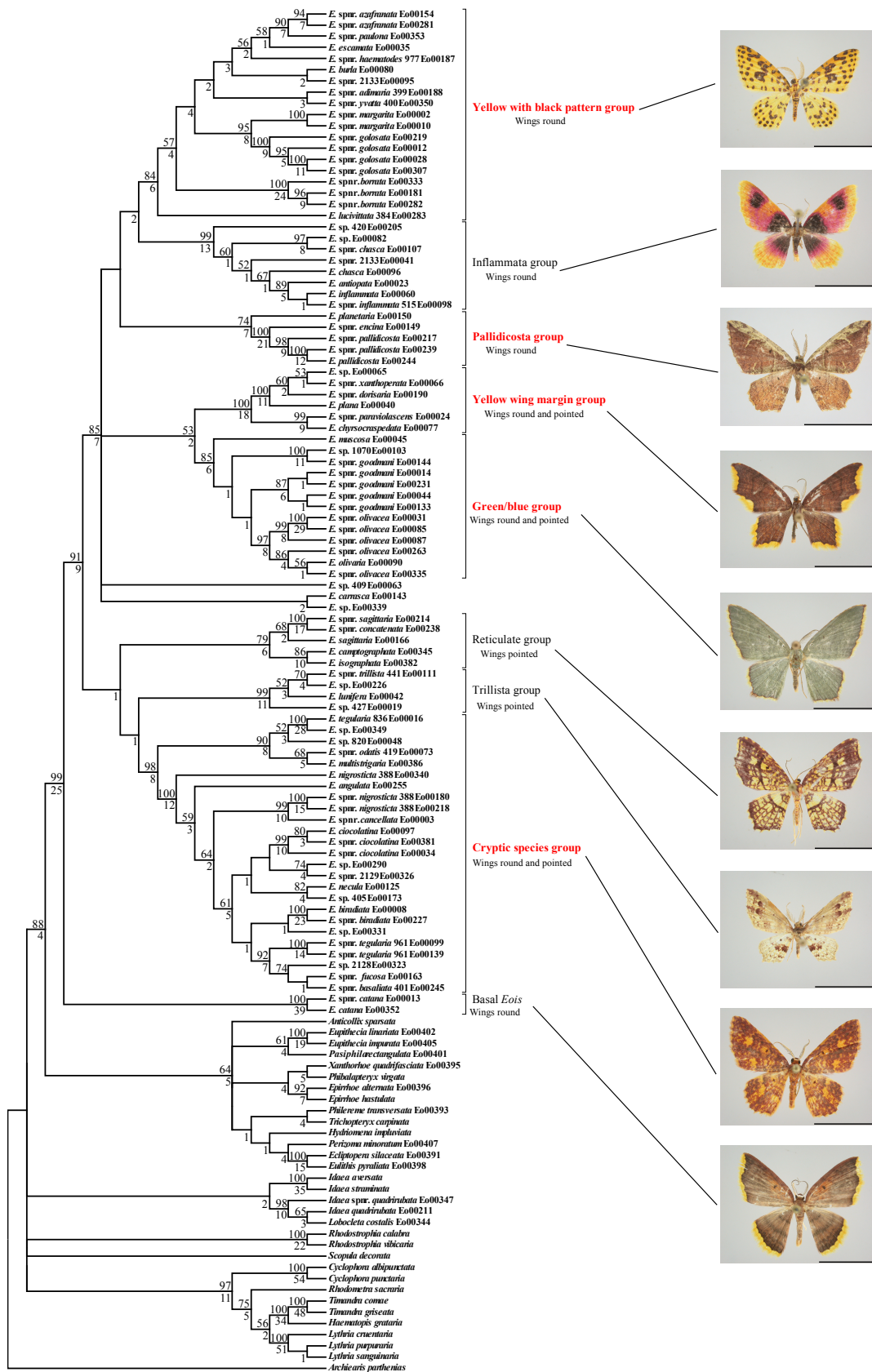
The origin of subclades within *Eois* was dated in the Oligocene and early Miocene. Most splits within subclades occurred throughout the Miocene with very few splits being dated in the Pliocene. Notably only one interspecific split was dated in the Quaternary period.

## **4. Discussion**

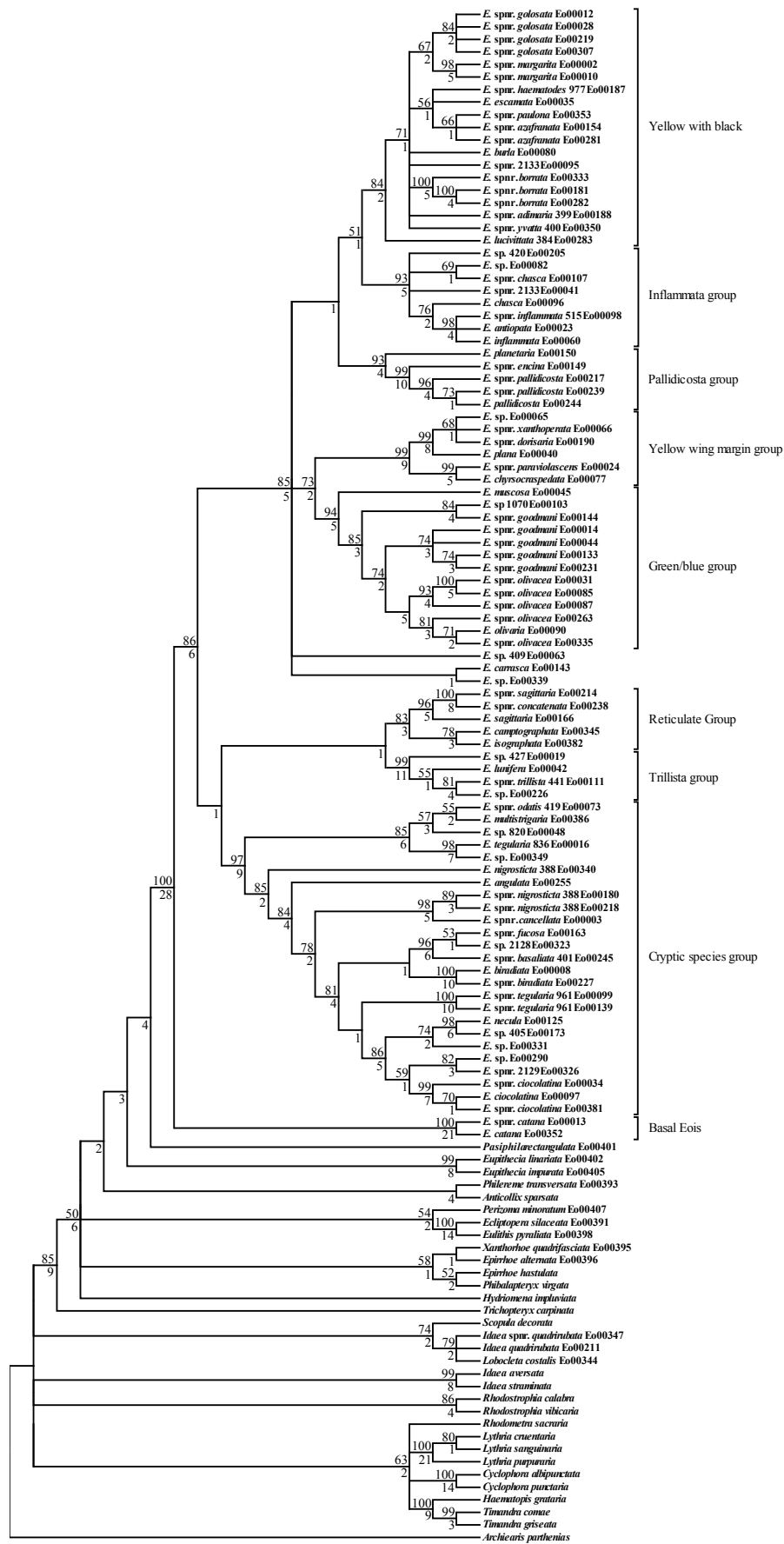
### *DNA barcoding and taxonomy*

With the discovery of 30 novel morphospecies the species count in one small area of rainforest in southern Ecuador rises from 99 to 129 in the genus *Eois* alone. The number rises further to 135 or even 138 when accepting the species recovered by threshold delimitation at 3% and 2%, respectively. This overall growth of the species list is quite remarkable, since earlier inventories were based on 3608 individuals of the genus *Eois* sampled during massive light-trapping campaigns at 39 sites (Brehm et al. 2005; Hilt et al. 2006). However, much of the material available for the sequence analysis came from quebrada forests which hold a distinct flora (Günter et al., 2008). This apparently also holds true for the geometrid genus *Eois*, whereas in the moth family Arctiidae, for example, rather few species were added to the local list by sampling in quebrada forests (Zimmermann, 2005). The discovery of 6-9 additional 'cryptic' new species by means of barcode analyses was not unexpected when compared to other recent studies of barcodes in tropical insect faunas (e.g. Condon et al., 2008; Smith et al., 2008). The amount of interspecific COI sequence divergence of the 6 additional species recognized by the 3% threshold is well within the range of divergence found between other, morphologically well defined, species within *Eois*. *Efl* $\alpha$  sequence divergences are consistent with the presence of separate species in all these cases. However, in the case of *E. spnr. biradiata* Sp008 the *Efl* $\alpha$  divergence is only 0.09% (1 base difference) which is markedly lower than in other cases with comparable COI distances. First data on genital morphology indicate that male genitalia provide no characters to distinguish between these critical species pairs (Strutzenberger, unpublished data). Analysis of female genitalia is troubled by the absence of female specimens in light-trap samples for most species. The fact that such high amounts of sequence divergence can be maintained in sympatric populations is a strong indicator for the presence of reproductive isolation. Yet, to conclusively support the species status of the newly recognized sequence types it will be necessary to supplement the evidence for these putative species with data on life histories, resource use or microdistribution.

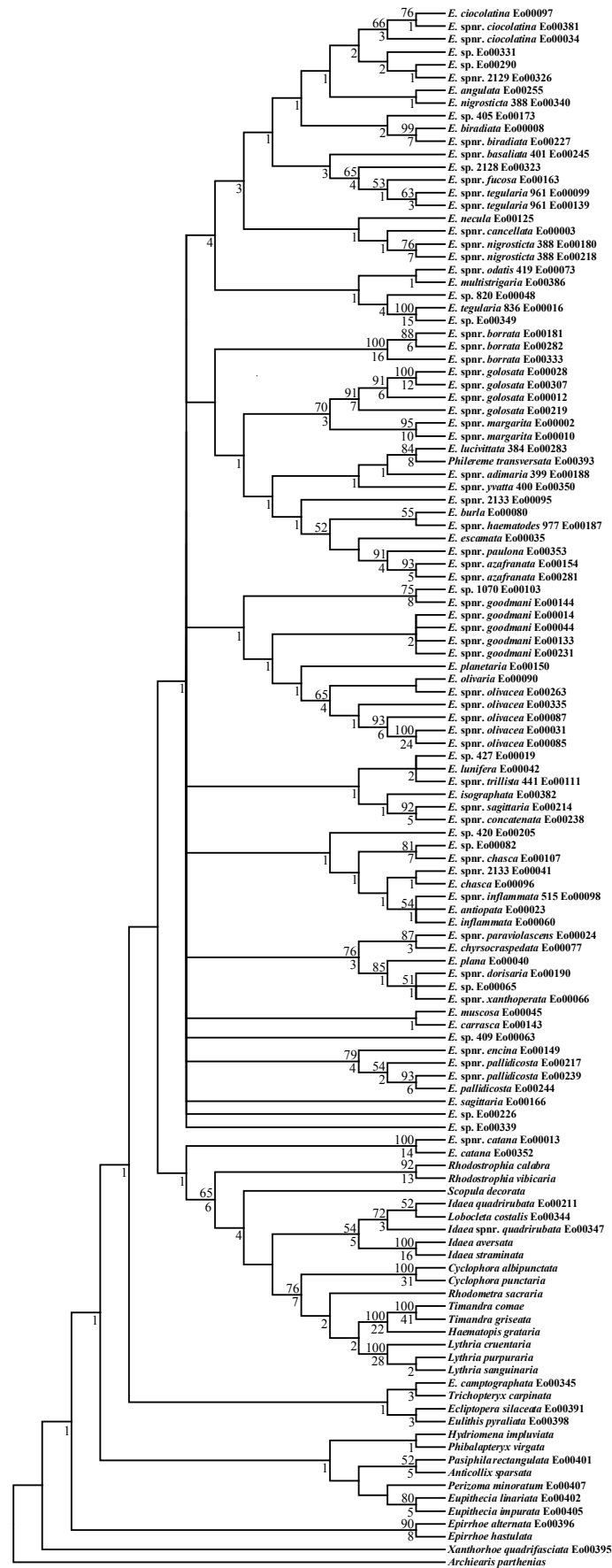
DNA barcoding performed badly when tested against morphological species which is not surprising in a group lacking proper taxonomic treatment. The finding that when using morphology-based species error is minimized at 3% as well as the fact that species delimitation at 2.8% would already result in a clear barcoding gap is in good agreement with early claims by proponents of DNA barcoding. A threshold value of 3% for the minimum sequence divergence between congeneric species enabled Hebert et al. (2003a) to correctly distinguish 98% of morphologically defined lepidopteran test species. This value has also been confirmed by Barrett and Hebert (2005) for arachnids, and Hebert et al. (2004a) found that a 2.7% threshold value for birds identifies 90% of the examined species. Setting the threshold at ten times the mean intraspecific divergence as proposed by Hebert et al. (2004a) for identification of potentially new species with minimal false positives would in the case of morphology-based species result in the threshold being set to 12.9% which would fail to correctly identify any of the included species. When the species resulting from delimitation at a threshold of 3% are used as reference, the threshold would be set to 3.7% corresponding to a total error rate of 12% including one false positive. When using species resulting from delimitation at a 2% threshold the threshold would be 3.2% which produces an error rate of 15%, all being false negatives. A threshold of ten times the mean



**Figure 5.** Maximum parsimony strict consensus tree of combined analysis with bootstrap support values indicated above branches and Bremer support values below branches. Clades with known Piperaceae feeders are marked in red. Photographs by Gunnar Brehm.



**Figure 6.** Maximum parsimony strict consensus tree based on Efl $\alpha$  sequences with bootstrap values indicated above branches and Bremer support values below branches.



**Figure 7.** Maximum parsimony strict consensus tree based on COI sequences with bootstrap values indicated above branches and Bremer support values below branches.

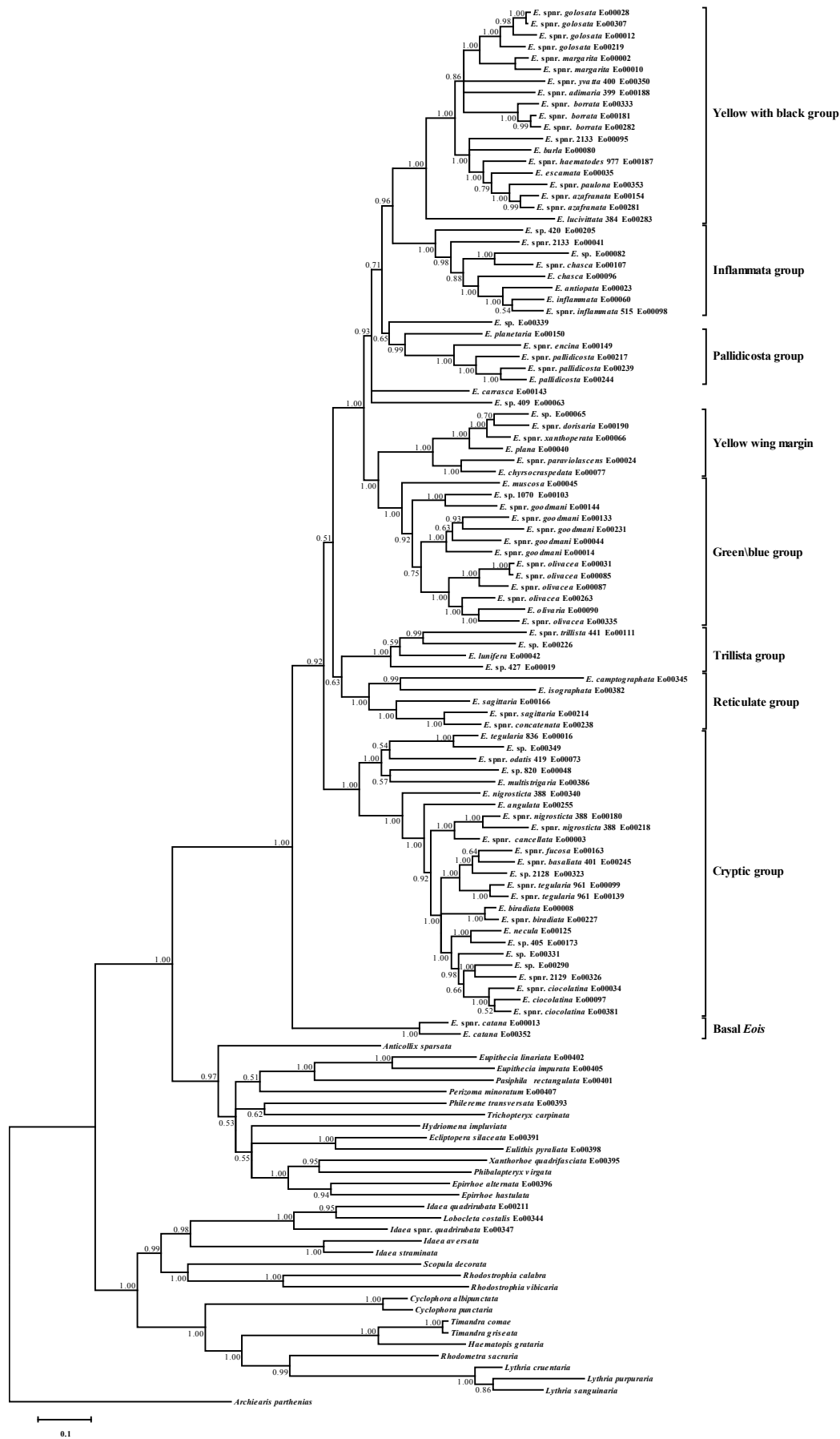


Figure 8. Bayesian 50% consensus tree from combined analysis. Scale bar is given as changes per site.

interspecific divergence does well in minimizing false positives but generates up to 100% false negatives as in case of morphology based species which is the most obvious scenario when screening for potential new species. Thus, in agreement with Meyer and Paulay (2005) we were unable to confirm the applicability of a general standard threshold defined in this way. In the present study a threshold of 2% has proven to be the most useful to screen for novel taxa.

Non-monophyly of species in morphological delimitation occurred in only two cases. Based on molecular data, both of these species are split into two distinct clades which most likely represent separate species. Accuracy of tree-based species identification is expected to be high in threshold based species as well as morphological species as in the latter only two cases of non monophyletic species could be detected and the former are monophyletic by definition. In the present analysis DNA barcoding proved to be a well suited tool to uncover potential shortcomings of traditional taxonomy. In this study the applicability of barcoding is likely to be greatly facilitated by the very limited geographic range of sampling. No intraspecific geographic variation can be expected to occur when all samples come from the same few square-kilometers. The DNA barcoding library presented here has already been successfully used to match caterpillars of *Eois* with species delimited by means of adult characters in six cases. DNA barcoding revealed that *E. ciocolatina* and *E. spnr. ciocolatina* feed on *Peperomia* instead of *Piper* unlike all other *Eois* investigated so far (Bodner pers. comm., 2008).

#### *Phylogenetic analysis*

The high number of trees found in parsimony analyses of the *Efl* $\alpha$  gene can be attributed to the lack of informative characters in recent species splits which can therefore not be resolved. On the other hand analysis of the COI gene was troubled by the high degree of homoplasy as expected from the Ti/Tv ratio plot. To avoid local optima it was necessary to search the tree space more extensively with COI data compared to combined analyses or those of the *Efl* $\alpha$  gene alone. Not all topologies of the same length were recovered for the COI dataset in the search for the most parsimonious tree. Additional trees of the same length were found during calculation of Bremer support values resulting in a Bremer support value of 0 for some nodes in the tree presented in Fig 6. Trees calculated from COI gene sequences are virtually unresolved except for sister species divergences. These trees are therefore not discussed any further. The significant result of the partition homogeneity test can be explained by the incomplete overlap of resolving power of the two genes as evident when examining the trees resulting from the two genes (Fig 6 and 7). The results of the combined analyses show that the number of most parsimonious trees is reduced to only 3. Consistency index and Retention index of combined trees are lower than for trees based on *Efl* $\alpha$  sequences, but higher than the ones for the trees based on the COI gene. Support values show a mixed picture when compared to the single gene trees as there are some nodes where support is higher whereas it is lower for other nodes but still acceptable in most cases (see also Table 6). The combined trees can therefore be taken as a compromise between resolution in older nodes and resolution in young nodes. This characteristic of the dataset does not allow any evaluation of potential conflict between mitochondrial and nuclear gene trees.

No clear trends in the evolution of morphological features could be recognized. However, all wing pattern types as defined from morphological inspection evolved only once with the possible exception of the 'Yellow wing margin' type. The members of the basalmost clade within *Eois* (*E. catana* + *E. spnr. catana*) closely resemble the wing pattern found in the 'Yellow wing margin' group. This may either be the plesiomorphic state for *Eois* or a case of convergent evolution (photographs are presented in Fig. 5). Wing shapes are variable as pointed and round wings are found in the same clade and even among sister species. Hence, although their monophyly has now been established by use of sequence data, the striking morphological diversity of Neotropical *Eois* moths deserves further study to unravel the underlying evolutionary dynamics. The timing of divergences within *Eois* is in contrast to the



notion that Neotropical biodiversity is a result of forest fragmentation caused by Pleistocene glacial cycles (Hooghiemstra and van der Hammen, 1998).

**Table 6.** Node based definitions for each subclade as well as for *Eois* as a whole are indicated along with support values for combined analysis and analysis of the *Efl $\alpha$*  gene.

Name	Definition	combined			Efl $\alpha$		
		Bootstrap	Bremer	Bayes	Bootstrap	Bremer	Bayes
Yellow with black pattern group	< <i>E. golosata</i> & <i>E. lucivittata</i>	84	6	1	84	2	1
Inflammata group	< <i>E. inflammata</i> & <i>E. sp. 420</i>	99	13	1	93	5	1
Pallidicosta group	< <i>E. pallidicosta</i> & <i>E. planetaria</i>	74	7	0.99	93	4	0.95
Yellow wing margin group	< <i>E. chrysocraspedata</i> & <i>E. xanthoperata</i>	100	18	1	99	9	1
Green/blue group	< <i>E. muscosa</i> & <i>E. olivacea</i>	85	6	1	94	5	1
Trillista group	< <i>E. trillista</i> & <i>E. sp 427</i>	99	11	1	99	11	1
Reticulate group	< <i>E. camptographata</i> & <i>E. concatenata</i>	79	6	1	83	3	1
Cryptic species group	< <i>E. ciocolatina</i> & <i>E. odatis</i>	98	8	1	97	9	0.99
<i>Eois</i>	< <i>E. catana</i> & <i>E. inflammata</i>	99	25	1	100	28	1

When the available foodplant records for Neotropical *Eois* (Dyer et al., 2008; Dyer and Gentry, 2002; Janzen and Hallwachs, 2005; Bodner, 2007; Bodner, pers. comm., 2008) are mapped on the tree (Fig 5) it becomes apparent that the host plant relationship with Piperaceae is widespread within *Eois*. Records are present for a number of subclades, namely the ‘Yellow with black pattern’ clade, the ‘*Pallidicosta* group’, the ‘Yellow wing margin group’ clade, the ‘Green/blue’ clade and the ‘Cryptic’ group which also contains the two taxa found to feed on *Peperomia* by Bodner (2008, pers. comm.). On the other hand the number of species and even more so the number of individuals recorded is highly skewed towards the ‘Cryptic’ species group whereas there are only one or a few records each for the other clades. At this stage it cannot be told whether this represents actual patterns of host plant relationships or is just an artifact of insufficient sampling. Dating of divergences shows that timing of major diversifications of *Piper* and *Peperomia* (Smith et al., 2008) is fully compatible with divergence times observed within *Eois*. Notably, the only host plant records of Asiatic representatives of the genus *Eois* refer to an entirely unrelated plant genus and family (*Mallotus*, Euphorbiaceae) (Singh, 1953). This opens up the question whether the affiliation with Piperaceae host plants is a derived trait within *Eois* that might have evolved only in those subclades that occur in the New World. Alternatively, the Piperaceae affiliation could represent the ancestral character state, and other host plants would then result from secondary shifts. These questions can only be tackled by including Old World representatives into phylogenetic studies and by assembling more complete host plant data.

Concerning the placement of *Eois* within the Larentiinae the only adequately supported placement we could recover shows *Eois* to be outside of all other included Larentiinae, among them two members of *Eupithecia*. We were unable to confirm the placement of Holloway (1997), who put *Eois* in the Eupitheciini, or present an alternative placement. In all trees distances to Eupitheciini were rather large, which would not support a close relationship between this enormously diverse tribe and *Eois*. However, rather few sequences are today available for species from the subfamily Larentiinae, and these are strongly biased towards Holarctic taxa, (Ounap, 2008). To settle the issue definitely, a more thorough sampling of larentiine taxa and a larger sequence dataset will be necessary.

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## Appendix

**Table A1.** List of all species recovered by morphological delimitation with the number of individuals per species and the range of intraspecific divergences indicated. Species newly recorded for the study area in Ecuador (RBSF) are printed in bold. Numeric species codes are given for reference to G. Brehm's database where applicable.

Species	Name	No. Ind.	Range of intraspecific distance
Sp001	<i>E. ciocolatina</i>	19	0 - 0.30%
<b>Sp002</b>	<b><i>E. spnr. ciocolatina</i></b>	7	0 - 0.45%
<b>Sp003</b>	<b><i>E. sp.</i></b>	5	0 - 0.74%
<b>Sp004</b>	<b><i>E. sp.</i></b>	2	0%
Sp005	<i>E. sp. 405</i>	2	0.74%
Sp006	<i>E. necula</i>	2	0%
Sp007	<i>E. biradiata</i>	6	0 - 3.35%
Sp008	included in Sp007		
<b>Sp009</b>	<b><i>E. spnr. nigrostricta</i> 388</b>	3	0 - 0.29%
<b>Sp010</b>	<b><i>E. spnr. fucosa</i></b>	7	0 - 0.31%
Sp011	<i>E. angulata</i>	2	0.45%
Sp012	<i>E. tegularia</i> 836	4	0.15 - 0.45%
Sp013	<i>E. spnr. odatis</i> 419	7	0 - 0.45%
Sp014	<i>E. sp.</i>	2	0.30%
Sp015	<i>E. sp. 427</i>	3	0.16 - 0.45%
<b>Sp016</b>	<b><i>E. spnr. trillista</i></b>	3	0 - 0.15%
Sp017	<i>E. lunifera</i>	8	0 - 0.15%
Sp018	<i>E. sp. 820</i>	6	0 - 0.75%
Sp019	<i>E. chasca</i>	16	0 - 1.35%
<b>Sp020</b>	<b><i>E. spnr. sp. 2133</i></b>	4	0 - 0.90%
Sp021	<i>E. spnr. inflammata</i> 515	3	0%
<b>Sp022</b>	<b><i>E. spnr. encina</i></b>	2	0%
<b>Sp023</b>	<b><i>E. spnr. pallidicosta</i></b>	2	0.15%
Sp024	<i>E. pallidicosta</i>	5	0 - 0.74%
Sp025	<i>E. muscosa</i>	6	0 - 0.89%
<b>Sp026</b>	<b><i>E. spnr. goodmani</i></b>	8	0 - 1.2%
<b>Sp027</b>	<b><i>E. spnr. goodmani</i></b>	5	0 - 6.36%
<b>Sp028</b>	<b><i>E. spnr. goodmani</i></b>	4	0%
<b>Sp029</b>	<b><i>E. spnr. goodmani</i></b>	4	0%
<b>Sp030</b>	<b><i>E. spnr. olivacea</i></b>	2	1.05%
<b>Sp031</b>	<b><i>E. spnr. olivacea</i></b>	23	0 - 6.87%
Sp032	included in Sp031		
Sp033	<i>E. chrysocraspedata</i>	24	0 - 1.41%
Sp034	<i>E. plana</i>	12	0 - 0.48%
Sp035	<i>E. dorisaria</i>	17	0 - 5.45%
Sp036	<i>E. xanthoperata</i>	2	0.16%
Sp037	included in Sp035		
Sp039	<i>E. catana</i>	2	0%
Sp040	<i>E. planetaria</i>	7	0 - 0.30%
Sp041	<i>E. lucivittata</i> 384	3	0.16 - 0.30%
Sp042	<i>E. spnr. azafanata</i>	8	0.15 - 3.90%
Sp043	included in Sp042		
Sp044	<i>E. escamata</i>	2	0.30%
Sp045	<i>E. burla</i>	7	0 - 0.59%
Sp046	<i>E. spnr. adimaria</i> 399	2	0%
Sp047	<i>E. spnr. borata</i>	3	0 - 0.15%
<b>Sp048</b>	<b><i>E. spnr. yvatta</i> 400</b>	2	0.60%
<b>Sp049</b>	<b><i>E. spnr. gotosata</i></b>	12	0 - 4.29%
Sp050	included in Sp049		
<b>Sp051</b>	<b><i>E. spnr. margarita</i></b>	20	0 - 4.49%
Sp052	included in Sp051		
Sp053	<i>E. sp. 409</i>	3	0.45 - 0.59%
<b>Sp054</b>	<b><i>E. spnr. borata</i></b>	6	0 - 1.11%
Eo00003	<i>E. spnr. cancellata</i>	1	0%
<b>Eo00013</b>	<b><i>E. spnr. catana</i></b>	1	0%
Eo00023	<i>E. antiopata</i>	1	0%
Eo00024	<i>E. paraviolascens</i>	1	0%
Eo00060	<i>E. inflammata</i>	1	0%
Eo00082	<i>E. sp.</i>	1	0%
Eo00090	<i>E. spnr. olivaria</i>	1	0%
<b>Eo00095</b>	<b><i>E. spnr. 2133</i></b>	1	0%
Eo00099	<i>E. spnr. tegularia</i> 961	1	0%
Eo00103	<i>E. sp. 1070</i>	1	0%
<b>Eo00107</b>	<b><i>E. spnr. chasca</i></b>	1	0%
Eo00139	<i>E. spnr. tegularia</i> 961	1	0%
Eo00143	<i>E. carrasca</i>	1	0%
Eo00144	<i>E. spnr. goodmani</i>	1	0%
Eo00166	<i>E. sagittaria</i>	1	0%
Eo00169	included in Sp026		
Eo00181	<i>E. spnr. borata</i>	1	0%
Eo00187	<i>E. spnr. haematodes</i> 977	1	0%
Eo00205	<i>E. sp. 420</i>	1	0%

Eo00214	<i>E. spnr. sagittaria</i>	1	0%
Eo00218	included in Sp009		
Eo00219	included in Sp049		
<b>Eo00238</b>	<b><i>E. spnr. concatenata</i></b>	1	0%
<b>Eo00239</b>	<b><i>E. spnr. pallidicosta</i></b>	1	0%
<b>Eo00245</b>	<b><i>E. spnr. basaliata 401</i></b>	1	0%
Eo00311	<i>E. sp. 382</i>	1	0%
Eo00323	<i>E. sp. 2128</i>	1	0%
<b>Eo00326</b>	<b><i>E. spnr. 2129</i></b>	1	0%
Eo00335	<i>E. spnr. olivacea</i>	1	0%
Eo00339	<i>E. sp.</i>	1	0%
Eo00340	<i>E. nigrosticta 388</i>	1	0%
Eo00345	<i>E. camptographata</i>	1	0%
<b>Eo00346</b>	<b><i>E. spnr. binaria</i></b>	1	0%
Eo00349	<i>E. sp.</i>	1	0%
<b>Eo00353</b>	<b><i>E. spnr. paulone</i></b>	1	0%
<b>Eo00381</b>	<b><i>E. spnr. cioccolatina</i></b>	1	0%

**Table A2.** List of all species recovered by 3% and 2% threshold delimitation with the number of individuals per species and the range of intraspecific divergences indicated. Species newly recorded for the study area in Ecuador (RBSF) are printed in bold. Numeric species codes are given for reference to G. Brehm's database where applicable.

2% THRESHOLD				3% THRESHOLD		
Species	Name	No. Ind.	Range of intraspec. distance	Name	No. Ind.	Range of intraspec. distance
Sp001	<i>E. cioccolatina</i>	19	0 - 0.30%	<i>E. cioccolatina</i>	19	0 - 0.30%
Sp002	<b><i>E. spnr. cioccolatina</i></b>	7	0 - 0.45%	<b><i>E. spnr. cioccolatina</i></b>	7	0 - 0.45%
Sp003	<b><i>E. sp.</i></b>	5	0 - 0.74%	<b><i>E. sp.</i></b>	5	0 - 0.74%
Sp004	<b><i>E. sp.</i></b>	2	0%	<b><i>E. sp.</i></b>	2	0%
Sp005	<i>E. sp. 405</i>	2	0.74%	<i>E. sp. 405</i>	2	0.74%
Sp006	<i>E. necula</i>	2	0%	<i>E. necula</i>	2	0%
Sp007	<i>E. biradiata</i>	4	0 - 0.61%	<i>E. biradiata</i>	6	0 - 3.34%
Sp008	<b><i>E. spnr. biradiata</i></b>	2	0.15%	included in Sp007		
Sp009	<b><i>E. spnr. nigrosticta 388</i></b>	2	0%	<b><i>E. spnr. nigrosticta 388</i></b>	3	0 - 2.88%
Sp010	<b><i>E. spnr. fucosa</i></b>	7	0 - 0.31%	<b><i>E. spnr. fucosa</i></b>	7	0 - 0.31%
Sp011	<i>E. angulata</i>	2	0.45%	<i>E. angulata</i>	2	0.45%
Sp012	<i>E. tegularia 836</i>	4	0.15 - 0.45%	<i>E. tegularia 836</i>	4	0.15 - 0.45%
Sp013	<i>E. spnr. odatis 419</i>	7	0 - 0.45%	<i>E. spnr. odatis 419</i>	7	0 - 0.45%
Sp014	<i>E. sp.</i>	2	0.30%	<i>E. sp.</i>	2	0.30%
Sp015	<i>E. sp. 427</i>	3	0.16 - 0.45%	<i>E. sp. 427</i>	3	0.16 - 0.45%
Sp016	<b><i>E. spnr. trillista</i></b>	3	0 - 0.15%	<b><i>E. spnr. trillista</i></b>	3	0 - 0.15%
Sp017	<i>E. lunifera</i>	8	0 - 0.15%	<i>E. lunifera</i>	8	0 - 0.15%
Sp018	<i>E. sp. 820</i>	6	0 - 0.75%	<i>E. sp. 820</i>	6	0 - 0.75%
Sp019	<i>E. chasca</i>	16	0 - 1.35%	<i>E. chasca</i>	16	0 - 1.35%
Sp020	<b><i>E. spnr. sp. 2133</i></b>	4	0 - 0.90%	<b><i>E. spnr. sp. 2133</i></b>	4	0 - 0.90%
Sp021	<i>E. spnr. inflammata 515</i>	3	0%	<i>E. spnr. inflammata 515</i>	3	0%
Sp022	<b><i>E. spnr. encina</i></b>	2	0%	<b><i>E. spnr. encina</i></b>	2	0%
Sp023	<b><i>E. spnr. pallidicosta</i></b>	2	0.15%	<b><i>E. spnr. pallidicosta</i></b>	2	0.15%
Sp024	<i>E. pallidicosta</i>	5	0 - 0.74%	<i>E. pallidicosta</i>	5	0 - 0.74%
Sp025	<i>E. muscosa</i>	6	0 - 0.89%	<i>E. muscosa</i>	6	0 - 0.89%
Sp026	<b><i>E. spnr. goodmani</i></b>	8	0 - 1.19%	<b><i>E. spnr. goodmani</i></b>	8	0 - 1.19%
Sp027	<b><i>E. spnr. goodmani</i></b>	4	0 - 0.15%	<b><i>E. spnr. goodmani</i></b>	4	0 - 0.15%
Sp028	<b><i>E. spnr. goodmani</i></b>	4	0%	<b><i>E. spnr. goodmani</i></b>	4	0%
Sp029	<b><i>E. spnr. goodmani</i></b>	4	0%	<b><i>E. spnr. goodmani</i></b>	4	0%
Sp030	<b><i>E. spnr. olivacea</i></b>	2	1.05%	<b><i>E. spnr. olivacea</i></b>	2	1.05%
Sp031	<b><i>E. spnr. olivacea</i></b>	9	0 - 0.89%	<b><i>E. spnr. olivacea</i></b>	9	0 - 0.89%
Sp032	<b><i>E. spnr. olivacea</i></b>	14	0 - 1.50%	<b><i>E. spnr. olivacea</i></b>	14	0 - 1.50%
Sp033	<i>E. chrysocraspedata</i>	24	0 - 1.41%	<i>E. chrysocraspedata</i>	24	0 - 1.41%
Sp034	<i>E. plana</i>	12	0 - 0.48%	<i>E. plana</i>	12	0 - 0.48%
Sp035	<b><i>E. sp.</i></b>	6	0%	<b><i>E. sp.</i></b>	6	0%
Sp036	<i>E. xanthoperata</i>	2	0.16%	<i>E. xanthoperata</i>	2	0.16%
Sp037	<i>E. dorisaria</i>	11	0 - 0.94%	<i>E. dorisaria</i>	11	0 - 0.94%
Sp039	<i>E. catana</i>	2	0%	<i>E. catana</i>	2	0%
Sp040	<i>E. planetaria</i>	7	0 - 0.30%	<i>E. planetaria</i>	7	0 - 0.30%
Sp041	<i>E. lucivittata 384</i>	3	0.16 - 0.30%	<i>E. lucivittata 384</i>	3	0.16 - 0.30%
Sp042	<i>E. spnr. azafanata</i>	4	0.17 - 0.79%	<i>E. spnr. azafanata</i>	8	0.17 - 3.90%
Sp043	<b><i>E. spnr. azafanata</i></b>	4	0.15 - 1.19%	included in Sp042		
Sp044	<i>E. escamata</i>	2	0.30%	<i>E. escamata</i>	2	0.30%
Sp045	<i>E. burla</i>	7	0 - 0.60%	<i>E. burla</i>	7	0 - 0.60%
Sp046	<i>E. spnr. adimaria 399</i>	2	0%	<i>E. spnr. adimaria 399</i>	2	0%
Sp047	<i>E. spnr. borrata</i>	3	0 - 0.15%	<i>E. spnr. borrata</i>	3	0 - 0.15%
Sp048	<b><i>E. spnr. yvatta 400</i></b>	2	0.60%	<b><i>E. spnr. yvatta 400</i></b>	2	0.60%
Sp049	<b><i>E. spnr. golosata</i></b>	7	0 - 0.61%	<b><i>E. spnr. golosata</i></b>	7	0 - 0.61%
Sp050	<b><i>E. spnr. golosata</i></b>	4	0 - 1.20%	<b><i>E. spnr. golosata</i></b>	4	0 - 1.20%
Sp051	<b><i>E. spnr. margarita</i></b>	4	0 - 0.16%	<b><i>E. spnr. margarita</i></b>	4	0 - 0.16%
Sp052	<b><i>E. spnr. margarita</i></b>	16	0 - 0.45%	<b><i>E. spnr. margarita</i></b>	16	0 - 0.45%
Sp053	<i>E. sp. 409</i>	3	0.46 - 0.59%	<i>E. sp. 409</i>	3	0.46 - 0.59%
Sp054	<b><i>E. spnr. borrata</i></b>	6	0 - 1.11%	<b><i>E. spnr. borrata</i></b>	6	0 - 1.11%
Eo00003	<i>E. spnr. cancellata</i>	1	0%	<i>E. spnr. cancellata</i>	1	0%
Eo00013	<b><i>E. spnr. catana</i></b>	1	0%	<b><i>E. spnr. catana</i></b>	1	0%
Eo00023	<i>E. antiopata</i>	1	0%	<i>E. antiopata</i>	1	0%

Eo00024	<i>E. paraviolascens</i>	1	0%	<i>E. paraviolascens</i>	1	0%
Eo00060	<i>E. inflammata</i>	1	0%	<i>E. inflammata</i>	1	0%
Eo00082	<i>E. sp.</i>	1	0%	<i>E. sp.</i>	1	0%
Eo00090	<i>E. spnr. olivaria</i>	1	0%	<i>E. spnr. olivaria</i>	1	0%
Eo00095	<b>E. spnr. 2133</b>	1	0%	<b>E. spnr. 2133</b>	1	0%
Eo00099	<i>E. spnr. tegularia 961</i>	1	0%	<i>E. spnr. tegularia 961</i>	1	0%
Eo00103	<i>E. sp. 1070</i>	1	0%	<i>E. sp. 1070</i>	1	0%
Eo00107	<b>E. spnr. chasca</b>	1	0%	<b>E. spnr. chasca</b>	1	0%
Eo00139	<i>E. spnr. tegularia 961</i>	1	0%	<i>E. spnr. tegularia 961</i>	1	0%
Eo00143	<i>E. carrasca</i>	1	0%	<i>E. carrasca</i>	1	0%
Eo00144	<i>E. spnr. goodmani</i>	1	0%	<i>E. spnr. goodmani</i>	1	0%
Eo00166	<i>E. sagittaria</i>	1	0%	<i>E. sagittaria</i>	1	0%
Eo00169	<b>E. spnr. goodmani</b>	1	0%	<b>E. spnr. goodmani</b>	1	0%
Eo00181	<i>E. borrata</i>	1	0%	<i>E. borrata</i>	1	0%
Eo00187	<i>E. spnr. haematodes 977</i>	1	0%	<i>E. spnr. haematodes 977</i>	1	0%
Eo00205	<i>E. sp. 420</i>	1	0%	<i>E. sp. 420</i>	1	0%
Eo00214	<b>E. spnr. sagittaria</b>	1	0%	<b>E. spnr. sagittaria</b>	1	0%
Eo00218	<b>E. spnr. nigrosticta 388</b>	1	0%	included in Sp009		
Eo00219	<b>E. spnr. golosata</b>	1	0%	<b>E. spnr. golosata</b>	1	0%
Eo00238	<b>E. spnr. concatenata</b>	1	0%	<b>E. spnr. concatenata</b>	1	0%
Eo00239	<b>E. spnr. pallidicosta</b>	1	0%	<b>E. spnr. pallidicosta</b>	1	0%
Eo00245	<b>E. spnr. basaliata 401</b>	1	0%	<b>E. spnr. basaliata 401</b>	1	0%
Eo00311	<i>E. sp. 382</i>	1	0%	<i>E. sp. 382</i>	1	0%
Eo00323	<i>E. sp. 2128</i>	1	0%	<i>E. sp. 2128</i>	1	0%
Eo00326	<b>E. spnr. 2129</b>	1	0%	<b>E. spnr. 2129</b>	1	0%
Eo00335	<i>E. spnr. olivacea</i>	1	0%	<i>E. spnr. olivacea</i>	1	0%
Eo00339	<i>E. sp.</i>	1	0%	<i>E. sp.</i>	1	0%
Eo00340	<i>E. nigrosticta 388</i>	1	0%	<i>E. nigrosticta 388</i>	1	0%
Eo00345	<i>E. camptographata</i>	1	0%	<i>E. camptographata</i>	1	0%
Eo00346	<b>E. spnr. binaria</b>	1	0%	<b>E. spnr. binaria</b>	1	0%
Eo00349	<i>E. sp.</i>	1	0%	<i>E. sp.</i>	1	0%
Eo00353	<b>E. spnr. paulone</b>	1	0%	<b>E. spnr. paulone</b>	1	0%
Eo00381	<b>E. spnr. ciocolatina</b>	1	0%	<b>E. spnr. ciocolatina</b>	1	0%

**Table A3.** Morphological delimitation: List of all individuals with their species assignment, length of barcoding sequence and collection site indicated. Numeric species codes are given for reference to G. Brehm's database where applicable.

Species	Code	Name	Length	Collection site
outgroup	Eo00052	<i>Lissochlora diarita</i>	676	Q3#1
outgroup	Eo00053	<i>Cargolia sp.</i>	676	Q3#1
outgroup	Eo00054	<i>Chloropteryx opalaria</i>	676	Q3#1
outgroup	Eo00055		676	Q3#1
outgroup	Eo00056	<i>Cargolia arana</i>	676	Q3#1
outgroup	Eo00057	<i>Cargolia arana</i>	676	Q3#1
outgroup	Eo00058	<i>Cargolia arana</i>	676	Q3#1
outgroup	Eo00059		676	Q3#1
outgroup	Eo00104	<i>Cyclophora dorsivena</i>	676	Q5#2
outgroup	Eo00134	<i>Dithecodes distracta</i>	676	Q2#3
outgroup	Eo00135	<i>Chloropteryx punctilinea</i>	676	Q2#3
outgroup	Eo00211	<i>Idaea quadrirubata</i>	676	Q3#3
outgroup	Eo00259	<i>Idaea recrinita</i>	637	Q5#3
outgroup	Eo00334	<i>Cyclophora sp.</i>	676	BC-IV
outgroup	Eo00344	<i>Lobocleta costalis</i>	676	BC-II
outgroup	Eo00347	<i>Idaea spnr. quadrirubata</i>	676	BC-II
outgroup	Eo00374	<i>Idaea quadrirubata</i>	650	Q3-1
single	Eo00003	<i>E. spnr. cancellata</i>	676	Q2#2
single	Eo00013	<i>E. spnr. catana</i>	676	Q3#3
single	Eo00023	<i>E. antiopata</i>	676	Q2#3
single	Eo00024	<i>E. paraviolascens</i>	676	Q2#3
single	Eo00060	<i>E. inflammata</i>	676	Q3#3
single	Eo00082	<i>E. sp.</i>	676	Q3#2
single	Eo00090	<i>E. spnr. olivaria</i>	676	Q2#1
single	Eo00095	<i>E. spnr. 2133</i>	658	Q5#3
single	Eo00099	<i>E. spnr. tegularia 961</i>	676	Q2#3
single	Eo00103	<i>E. sp. 1070</i>	676	Q5#2
single	Eo00107	<i>E. spnr. chasca</i>	676	Q5#3
single	Eo00139	<i>E. spnr. tegularia 961</i>	676	Q2#3
single	Eo00143	<i>E. carrasca</i>	676	Q5#2
single	Eo00144	<i>E. spnr. goodmani</i>	676	Q3#2
single	Eo00166	<i>E. sagittaria</i>	676	Q2#3
single	Eo00181	<i>E. spnr. borrata</i>	676	Q2#3
single	Eo00187	<i>E. spnr. haematodes 977</i>	676	Q5#3
single	Eo00205	<i>E. sp. 420</i>	676	Q3#1
single	Eo00214	<i>E. spnr. sagittaria</i>	676	Q3#3
single	Eo00238	<i>E. spnr. concatenata</i>	676	Q5#2
single	Eo00239	<i>E. spnr. pallidicosta</i>	676	Q5#2
single	Eo00245	<i>E. spnr. basaliata 401</i>	676	Q5#3
single	Eo00311	<i>E. sp. 382</i>	676	Q2#3
single	Eo00323	<i>E. sp. 2128</i>	650	Q3#2
single	Eo00326	<i>E. spnr. 2129</i>	676	Q2#3
single	Eo00335	<i>E. spnr. olivacea</i>	676	BC-IV



single	Eo00339	<i>E. sp.</i>	676	BC-I
single	Eo00340	<i>E. nigrosticta</i> 388	676	BC-I
single	Eo00345	<i>E. camptographata</i>	676	BC-II
single	Eo00346	<i>E. spnr. binaria</i>	676	BC-II
single	Eo00349	<i>E. sp.</i>	676	BC-VI
single	Eo00353	<i>E. spnr. paulone</i>	676	4a
single	Eo00381	<i>E. spnr. ciocolatina</i>	650	4a
single	Eo00385	<i>E. parva</i>	676	1685
single	Eo00386	<i>E. multistrigaria</i>	676	1686
Sp001	Eo00020	<i>E. ciocolatina</i>	676	Q3#1
Sp001	Eo00022	<i>E. ciocolatina</i>	676	Q2#3
Sp001	Eo00032	<i>E. ciocolatina</i>	676	Q2#2
Sp001	Eo00064	<i>E. ciocolatina</i>	676	Q2#2
Sp001	Eo00079	<i>E. ciocolatina</i>	676	Q2#2
Sp001	Eo00088	<i>E. ciocolatina</i>	676	Q2#3
Sp001	Eo00097	<i>E. ciocolatina</i>	676	Q2#3
Sp001	Eo00100	<i>E. ciocolatina</i>	676	Q2#3
Sp001	Eo00101	<i>E. ciocolatina</i>	650	Q2#3
Sp001	Eo00116	<i>E. ciocolatina</i>	676	Q2#3
Sp001	Eo00142	<i>E. ciocolatina</i>	676	Q5#2
Sp001	Eo00158	<i>E. ciocolatina</i>	676	Q3#2
Sp001	Eo00165	<i>E. ciocolatina</i>	676	Q2#2
Sp001	Eo00168	<i>E. ciocolatina</i>	676	Q5#3
Sp001	Eo00177	<i>E. ciocolatina</i>	676	Q2#3
Sp001	Eo00351	<i>E. ciocolatina</i>	676	4a
Sp001	Eo00366	<i>E. ciocolatina</i>	676	Q3-1
Sp001	Eo00372	<i>E. ciocolatina</i>	676	Q3-1
Sp001	Eo00376	<i>E. ciocolatina</i>	676	Q3-1
Sp002	Eo00034	<i>E. spnr. ciocolatina</i>	676	Q2#2
Sp002	Eo00043	<i>E. spnr. ciocolatina</i>	676	Q2#3
Sp002	Eo00102	<i>E. spnr. ciocolatina</i>	676	Q2#3
Sp002	Eo00153	<i>E. spnr. ciocolatina</i>	676	Q2#3
Sp002	Eo00176	<i>E. spnr. ciocolatina</i>	676	Q2#3
Sp002	Eo00210	<i>E. spnr. ciocolatina</i>	676	Q3#1
Sp002	Eo00373	<i>E. spnr. ciocolatina</i>	676	Q3-1
Sp003	Eo00269	<i>E. sp.</i>	676	Q3#3
Sp003	Eo00273	<i>E. sp.</i>	658	Q3#3
Sp003	Eo00278	<i>E. sp.</i>	676	Q3#3
Sp003	Eo00290	<i>E. sp.</i>	676	Q3#3
Sp003	Eo00316	<i>E. sp.</i>	676	Q3#3
Sp004	Eo00129	<i>E. sp.</i>	676	Q2#3
Sp004	Eo00331	<i>E. sp.</i>	676	Q5#2
Sp005	Eo00173	<i>E. sp. 405</i>	676	Q3#3
Sp005	Eo00261	<i>E. sp. 405</i>	676	Q5#3
Sp006	Eo00062	<i>E. necula</i>	676	Q3#3
Sp006	Eo00125	<i>E. necula</i>	676	Q3#3
Sp007	Eo00008	<i>E. biradiata</i>	676	Q3#3
Sp007	Eo00227	<i>E. biradiata</i>	676	Q3#1
Sp007	Eo00272	<i>E. biradiata</i>	658	Q3#3
Sp007	Eo00286	<i>E. biradiata</i>	676	Q3#3
Sp007	Eo00304	<i>E. biradiata</i>	676	Q3#3
Sp007	Eo00365	<i>E. biradiata</i>	676	Q3-1
Sp009	Eo00180	<i>E. spnr. nigrosticta</i> 388	676	Q2#3
Sp009	Eo00207	<i>E. spnr. nigrosticta</i> 388	676	Q3#1
Sp009	Eo00218	<i>E. spnr. nigrosticta</i> 388	676	Q5#3
Sp010	Eo00163	<i>E. spnr. fucosa</i>	676	Q3#1
Sp010	Eo00186	<i>E. spnr. fucosa</i>	676	Q5#3
Sp010	Eo00197	<i>E. spnr. fucosa</i>	676	Q3#1
Sp010	Eo00315	<i>E. spnr. fucosa</i>	676	Q5#1
Sp010	Eo00319	<i>E. spnr. fucosa</i>	676	Q5#1
Sp010	Eo00324	<i>E. spnr. fucosa</i>	656	Q2#2
Sp010	Eo00325	<i>E. spnr. fucosa</i>	676	Q2#2
Sp011	Eo00255	<i>E. angulata</i>	676	Q5#1
Sp011	Eo00341	<i>E. angulata</i>	676	BC-I
Sp012	Eo00016	<i>E. tegularia</i> 836	676	Q3#1
Sp012	Eo00072	<i>E. tegularia</i> 836	676	Q5#2
Sp012	Eo00109	<i>E. tegularia</i> 836	676	Q2#3
Sp012	Eo00309	<i>E. tegularia</i> 836	676	Q5#1
Sp013	Eo00073	<i>E. spnr. odatis</i> 419	676	Q3#3
Sp013	Eo00114	<i>E. spnr. odatis</i> 419	676	Q2#3
Sp013	Eo00115	<i>E. spnr. odatis</i> 419	676	Q2#3
Sp013	Eo00141	<i>E. spnr. odatis</i> 419	676	Q2#3
Sp013	Eo00265	<i>E. spnr. odatis</i> 419	676	Q5#3
Sp013	Eo00313	<i>E. spnr. odatis</i> 419	635	Q2#3
Sp013	Eo00332	<i>E. spnr. odatis</i> 419	676	Q5#2
Sp014	Eo00226	<i>E. sp.</i>	676	Q3#1
Sp014	Eo00362	<i>E. sp.</i>	676	Q3-1
Sp015	Eo00019	<i>E. sp. 427</i>	676	Q3#1
Sp015	Eo00192	<i>E. sp. 427</i>	676	Q3#1
Sp015	Eo00308	<i>E. sp. 427</i>	647	Q5#2
Sp016	Eo00111	<i>E. trillista</i>	676	Q2#3
Sp016	Eo00112	<i>E. trillista</i>	676	Q2#3
Sp016	Eo00119	<i>E. trillista</i>	676	Q2#3
Sp017	Eo00001	<i>E. lunifera</i>	676	Q2#3

Sp017	Eo00042	<i>E. lunifera</i>	676	Q2#3
Sp017	Eo00120	<i>E. lunifera</i>	676	Q2#3
Sp017	Eo00198	<i>E. lunifera</i>	676	Q3#1
Sp017	Eo00243	<i>E. lunifera</i>	658	Q5#2
Sp017	Eo00256	<i>E. lunifera</i>	676	Q5#3
Sp017	Eo00257	<i>E. lunifera</i>	676	Q5#3
Sp017	Eo00296	<i>E. lunifera</i>	676	Q5#1
Sp018	Eo00025	<i>E. sp. 820</i>	676	Q2#3
Sp018	Eo00048	<i>E. sp. 820</i>	676	Q3#3
Sp018	Eo00131	<i>E. sp. 820</i>	676	Q2#3
Sp018	Eo00155	<i>E. sp. 820</i>	676	Q2#3
Sp018	Eo00295	<i>E. sp. 820</i>	676	Q3#3
Sp018	Eo00317	<i>E. sp. 820</i>	676	Q5#2
Sp019	Eo00004	<i>E. chasca</i>	676	Q3#2
Sp019	Eo00036	<i>E. chasca</i>	676	Q2#1
Sp019	Eo00050	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00096	<i>E. chasca</i>	676	Q5#3
Sp019	Eo00124	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00146	<i>E. chasca</i>	676	Q3#2
Sp019	Eo00161	<i>E. chasca</i>	676	Q3#1
Sp019	Eo00170	<i>E. chasca</i>	661	Q5#3
Sp019	Eo00175	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00178	<i>E. chasca</i>	676	Q2#3
Sp019	Eo00215	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00251	<i>E. chasca</i>	676	Q5#1
Sp019	Eo00258	<i>E. chasca</i>	676	Q5#3
Sp019	Eo00292	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00360	<i>E. chasca</i>	676	Q3-1
Sp019	Eo00368	<i>E. chasca</i>	676	Q3-1
Sp020	Eo00041	<i>E. spnr. sp. 2133</i>	676	Q2#3
Sp020	Eo00069	<i>E. spnr. sp. 2133</i>	676	Q2#2
Sp020	Eo00086	<i>E. spnr. sp. 2133</i>	676	Q2#3
Sp020	Eo00270	<i>E. spnr. sp. 2133</i>	676	Q3#3
Sp021	Eo00098	<i>E. spnr. inflammata 515</i>	676	Q2#3
Sp021	Eo00206	<i>E. spnr. inflammata 515</i>	676	Q3#1
Sp021	Eo00230	<i>E. spnr. inflammata 515</i>	676	Q5#1
Sp022	Eo00149	<i>E. spnr. encina</i>	676	Q3#2
Sp022	Eo00262	<i>E. spnr. encina</i>	676	Q2#1
Sp023	Eo00108	<i>E. spnr. pallidicosta</i>	658	Q5#3
Sp023	Eo00217	<i>E. spnr. pallidicosta</i>	676	Q5#3
Sp024	Eo00110	<i>E. pallidicosta</i>	676	Q2#3
Sp024	Eo00138	<i>E. pallidicosta</i>	676	Q2#1
Sp024	Eo00242	<i>E. pallidicosta</i>	676	Q5#2
Sp024	Eo00244	<i>E. pallidicosta</i>	676	Q5#3
Sp024	Eo00329	<i>E. pallidicosta</i>	676	Q3#3
Sp025	Eo00045	<i>E. muscosa</i>	676	Q2#3
Sp025	Eo00136	<i>E. muscosa</i>	676	Q2#2
Sp025	Eo00140	<i>E. muscosa</i>	676	Q2#3
Sp025	Eo00162	<i>E. muscosa</i>	676	Q3#1
Sp025	Eo00204	<i>E. muscosa</i>	676	Q3#1
Sp025	Eo00254	<i>E. muscosa</i>	676	Q5#1
Sp026	Eo00123	<i>E. spnr. goodmani</i>	676	Q5#3
Sp026	Eo00132	<i>E. spnr. goodmani</i>	676	Q2#3
Sp026	Eo00133	<i>E. spnr. goodmani</i>	676	Q2#3
Sp026	Eo00185	<i>E. spnr. goodmani</i>	676	Q5#3
Sp026	Eo00246	<i>E. spnr. goodmani</i>	676	Q5#3
Sp026	Eo00298	<i>E. spnr. goodmani</i>	650	Q5#1
Sp026	Eo00300	<i>E. spnr. goodmani</i>	676	Q5#1
Sp026	Eo00322	<i>E. spnr. goodmani</i>	676	Q3#2
Sp027	Eo00044	<i>E. spnr. goodmani</i>	676	Q2#3
Sp027	Eo00074	<i>E. spnr. goodmani</i>	676	Q3#3
Sp027	Eo00169	<i>E. spnr. goodmani</i>	676	Q5#3
Sp027	Eo00179	<i>E. spnr. goodmani</i>	676	Q2#3
Sp027	Eo00183	<i>E. spnr. goodmani</i>	676	Q5#3
Sp028	Eo00014	<i>E. spnr. goodmani</i>	676	Q3#3
Sp028	Eo00274	<i>E. spnr. goodmani</i>	676	Q3#3
Sp028	Eo00289	<i>E. spnr. goodmani</i>	676	Q3#3
Sp028	Eo00294	<i>E. spnr. goodmani</i>	676	Q3#3
Sp029	Eo00209	<i>E. spnr. goodmani</i>	676	Q3#1
Sp029	Eo00228	<i>E. spnr. goodmani</i>	676	Q3#1
Sp029	Eo00231	<i>E. spnr. goodmani</i>	676	Q5#1
Sp029	Eo00248	<i>E. spnr. goodmani</i>	676	Q5#1
Sp030	Eo00263	<i>E. spnr. olivacea</i>	676	Q2#3
Sp030	Eo00356	<i>E. spnr. olivacea</i>	676	4a
Sp031	Eo00031	<i>E. spnr. olivacea</i>	676	Q2#1
Sp031	Eo00047	<i>E. spnr. olivacea</i>	676	Q3#3
Sp031	Eo00085	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00087	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00089	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00105	<i>E. spnr. olivacea</i>	676	Q5#2
Sp031	Eo00106	<i>E. spnr. olivacea</i>	676	Q5#2
Sp031	Eo00113	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00117	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00127	<i>E. spnr. olivacea</i>	676	Q2#3

Sp031	Eo00137	<i>E. spnr. olivacea</i>	676	Q2#1
Sp031	Eo00171	<i>E. spnr. olivacea</i>	676	Q5#3
Sp031	Eo00182	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00200	<i>E. spnr. olivacea</i>	676	Q5#3
Sp031	Eo00213	<i>E. spnr. olivacea</i>	676	Q3#1
Sp031	Eo00223	<i>E. spnr. olivacea</i>	676	Q3#1
Sp031	Eo00241	<i>E. spnr. olivacea</i>	676	Q5#2
Sp031	Eo00252	<i>E. spnr. olivacea</i>	676	Q5#1
Sp031	Eo00260	<i>E. spnr. olivacea</i>	676	Q5#3
Sp031	Eo00264	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00301	<i>E. spnr. olivacea</i>	676	Q5#1
Sp031	Eo00303	<i>E. spnr. olivacea</i>	676	Q5#1
Sp031	Eo00363	<i>E. spnr. olivacea</i>	676	Q3-1
Sp033	Eo00030	<i>E. chrysocraspedata</i>	676	Q2#1
Sp033	Eo00033	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00038	<i>E. chrysocraspedata</i>	676	Q2#1
Sp033	Eo00067	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00068	<i>E. chrysocraspedata</i>	650	Q2#2
Sp033	Eo00077	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00078	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00145	<i>E. chrysocraspedata</i>	676	Q3#2
Sp033	Eo00147	<i>E. chrysocraspedata</i>	647	Q3#2
Sp033	Eo00148	<i>E. chrysocraspedata</i>	676	Q3#2
Sp033	Eo00160	<i>E. chrysocraspedata</i>	676	Q3#2
Sp033	Eo00164	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00196	<i>E. chrysocraspedata</i>	676	Q3#1
Sp033	Eo00201	<i>E. chrysocraspedata</i>	676	Q5#3
Sp033	Eo00202	<i>E. chrysocraspedata</i>	653	Q5#3
Sp033	Eo00208	<i>E. chrysocraspedata</i>	676	Q3#1
Sp033	Eo00221	<i>E. chrysocraspedata</i>	676	Q3#1
Sp033	Eo00222	<i>E. chrysocraspedata</i>	676	Q2#1
Sp033	Eo00229	<i>E. chrysocraspedata</i>	676	Q3#1
Sp033	Eo00247	<i>E. chrysocraspedata</i>	635	Q5#1
Sp033	Eo00250	<i>E. chrysocraspedata</i>	676	Q5#1
Sp033	Eo00361	<i>E. chrysocraspedata</i>	676	Q3-1
Sp033	Eo00369	<i>E. chrysocraspedata</i>	676	Q3-1
Sp033	Eo00377	<i>E. chrysocraspedata</i>	676	Q3-1
Sp034	Eo00017	<i>E. plana</i>	663	Q3#1
Sp034	Eo00029	<i>E. plana</i>	676	Q3#2
Sp034	Eo00039	<i>E. plana</i>	676	Q2#1
Sp034	Eo00040	<i>E. plana</i>	676	Q3#1
Sp034	Eo00081	<i>E. plana</i>	644	Q3#2
Sp034	Eo00083	<i>E. plana</i>	676	Q3#2
Sp034	Eo00091	<i>E. plana</i>	676	Q2#1
Sp034	Eo00094	<i>E. plana</i>	676	Q5#3
Sp034	Eo00193	<i>E. plana</i>	676	Q3#1
Sp034	Eo00195	<i>E. plana</i>	676	Q3#1
Sp034	Eo00203	<i>E. plana</i>	676	Q5#3
Sp034	Eo00225	<i>E. plana</i>	676	Q3#1
Sp035	Eo00018	<i>E. dorisaria</i>	651	Q3#1
Sp035	Eo00065	<i>E. sp.</i>	676	Q2#2
Sp035	Eo00070	<i>E. sp.</i>	676	Q5#2
Sp035	Eo00184	<i>E. sp.</i>	676	Q5#3
Sp035	Eo00190	<i>E. dorisaria</i>	676	Q2#1
Sp035	Eo00191	<i>E. dorisaria</i>	676	Q2#1
Sp035	Eo00194	<i>E. sp.</i>	676	Q3#1
Sp035	Eo00224	<i>E. sp.</i>	676	Q3#1
Sp035	Eo00232	<i>E. dorisaria</i>	676	Q5#1
Sp035	Eo00233	<i>E. dorisaria</i>	676	Q5#1
Sp035	Eo00237	<i>E. dorisaria</i>	676	Q5#1
Sp035	Eo00253	<i>E. dorisaria</i>	676	Q5#1
Sp035	Eo00358	<i>E. dorisaria</i>	676	Q3-1
Sp035	Eo00359	<i>E. dorisaria</i>	676	Q3-1
Sp035	Eo00364	<i>E. sp.</i>	676	Q3-1
Sp035	Eo00371	<i>E. dorisaria</i>	676	Q3-1
Sp035	Eo00375	<i>E. dorisaria</i>	676	Q3-1
Sp036	Eo00027	<i>E. xanthoperata</i>	650	Q3#2
Sp036	Eo00066	<i>E. xanthoperata</i>	676	Q2#2
Sp039	Eo00338	<i>E. catana</i>	676	BC-1
Sp039	Eo00352	<i>E. catana</i>	676	4a
Sp040	Eo00005	<i>E. planetaria</i>	676	Q3#3
Sp040	Eo00093	<i>E. planetaria</i>	676	Q2#3
Sp040	Eo00118	<i>E. planetaria</i>	676	Q3#3
Sp040	Eo00126	<i>E. planetaria</i>	676	Q3#3
Sp040	Eo00150	<i>E. planetaria</i>	676	Q3#2
Sp040	Eo00152	<i>E. planetaria</i>	676	Q2#3
Sp040	Eo00299	<i>E. planetaria</i>	676	Q5#1
Sp041	Eo00283	<i>E. lucivittata</i> 384	676	Q3#3
Sp041	Eo00379	<i>E. lucivittata</i> 384	676	4a
Sp041	Eo00380	<i>E. lucivittata</i> 384	651	4a
Sp042	Eo00154	<i>E. spnr. azafanata</i>	658	Q2#3
Sp042	Eo00167	<i>E. spnr. azafanata</i>	650	Q5#3
Sp042	Eo00199	<i>E. spnr. azafanata</i>	676	Q5#3
Sp042	Eo00266	<i>E. spnr. azafanata</i>	635	Q5#3

Sp042	Eo00281	<i>E. spnr. azafranata</i>	676	Q3#3
Sp042	Eo00291	<i>E. spnr. azafranata</i>	676	Q3#3
Sp042	Eo00310	<i>E. spnr. azafranata</i>	676	Q2#3
Sp042	Eo00312	<i>E. spnr. azafranata</i>	676	Q2#3
Sp044	Eo00026	<i>E. escamata</i>	676	Q2#3
Sp044	Eo00035	<i>E. escamata</i>	676	Q2#2
Sp045	Eo00006	<i>E. burla</i>	676	Q3#3
Sp045	Eo00061	<i>E. burla</i>	650	Q3#3
Sp045	Eo00080	<i>E. burla</i>	676	Q2#2
Sp045	Eo00084	<i>E. burla</i>	674	Q2#3
Sp045	Eo00121	<i>E. burla</i>	676	Q5#3
Sp045	Eo00156	<i>E. burla</i>	664	Q2#3
Sp045	Eo00343	<i>E. burla</i>	676	KP1-V1
Sp046	Eo00188	<i>E. spnr. adimaria 399</i>	676	Q5#3
Sp046	Eo00287	<i>E. spnr. adimaria 399</i>	676	Q3#3
Sp047	Eo00189	<i>E. spnr. borrata</i>	676	Q5#3
Sp047	Eo00333	<i>E. spnr. borrata</i>	676	Q5#2
Sp047	Eo00388	<i>E. spnr. borrata</i>	676	Q5#1
Sp048	Eo00348	<i>E. spnr. yvatta 400</i>	676	BC-V1
Sp048	Eo00350	<i>E. spnr. yvatta 400</i>	676	BC-V1
Sp049	Eo00012	<i>E. spnr. golosata</i>	676	Q3#3
Sp049	Eo00028	<i>E. spnr. golosata</i>	676	Q3#2
Sp049	Eo00157	<i>E. spnr. golosata</i>	676	Q2#3
Sp049	Eo00159	<i>E. spnr. golosata</i>	658	Q3#2
Sp049	Eo00219	<i>E. spnr. golosata</i>	676	Q3#2
Sp049	Eo00240	<i>E. spnr. golosata</i>	676	Q5#2
Sp049	Eo00271	<i>E. spnr. golosata</i>	658	Q3#3
Sp049	Eo00288	<i>E. spnr. golosata</i>	676	Q3#3
Sp049	Eo00305	<i>E. spnr. golosata</i>	676	Q3#3
Sp049	Eo00307	<i>E. spnr. golosata</i>	676	Q5#2
Sp049	Eo00321	<i>E. spnr. golosata</i>	676	Q3#2
Sp049	Eo00330	<i>E. spnr. golosata</i>	676	Q3#3
Sp051	Eo00002	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00007	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00009	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00010	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00011	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00037	<i>E. spnr. margarita</i>	665	Q2#1
Sp051	Eo00049	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00051	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00075	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00076	<i>E. spnr. margarita</i>	652	Q3#3
Sp051	Eo00151	<i>E. spnr. margarita</i>	676	Q2#3
Sp051	Eo00172	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00212	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00220	<i>E. spnr. margarita</i>	676	Q3#2
Sp051	Eo00236	<i>E. spnr. margarita</i>	658	Q5#1
Sp051	Eo00268	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00285	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00302	<i>E. spnr. margarita</i>	676	Q5#1
Sp051	Eo00355	<i>E. spnr. margarita</i>	676	4a
Sp051	Eo00378	<i>E. spnr. margarita</i>	676	4a
Sp053	Eo00021	<i>E. sp. 409</i>	676	Q3#1
Sp053	Eo00063	<i>E. sp. 409</i>	676	Q2#2
Sp053	Eo00128	<i>E. sp. 409</i>	676	Q2#3
Sp054	Eo00071	<i>E. spnr. borrata</i>	636	Q5#2
Sp054	Eo00092	<i>E. spnr. borrata</i>	676	Q2#3
Sp054	Eo00130	<i>E. spnr. borrata</i>	635	Q2#3
Sp054	Eo00282	<i>E. spnr. borrata</i>	658	Q3#3
Sp054	Eo00284	<i>E. spnr. borrata</i>	658	Q3#3
Sp054	Eo00327	<i>E. spnr. borrata</i>	635	Q2#3
unknown	Eo00342		635	BC-I

**Table A4.** 3% threshold delimitation: List of all individuals with their species assignment, length of barcoding sequence and collection site indicated. Numeric species codes are given for reference to G. Brehm's database where applicable.

Species	Code	Name	Length	Collection site
outgroup	Eo00052	<i>Lissochlora diarita</i>	676	Q3#1
outgroup	Eo00053	<i>Cargolia</i> sp.	676	Q3#1
outgroup	Eo00054	<i>Chloropteryx opalaria</i>	676	Q3#1
outgroup	Eo00055		676	Q3#1
outgroup	Eo00056	<i>Cargolia arana</i>	676	Q3#1
outgroup	Eo00057	<i>Cargolia arana</i>	676	Q3#1
outgroup	Eo00058	<i>Cargolia arana</i>	676	Q3#1
outgroup	Eo00059		676	Q3#1
outgroup	Eo00104	<i>Cyclophora dorsivena</i>	676	Q5#2
outgroup	Eo00134	<i>Dithecodes distracta</i>	676	Q2#3
outgroup	Eo00135	<i>Chloropteryx punctilinea</i>	676	Q2#3
outgroup	Eo00211	<i>Idaea quadrirubata</i>	676	Q3#3
outgroup	Eo00259	<i>Idaea recrinita</i>	637	Q5#3
outgroup	Eo00334	<i>Cyclophora</i> sp.	676	BC-IV

outgroup	Eo00344	<i>Lobocleta costalis</i>	676	BC-II
outgroup	Eo00347	<i>Idae</i> spnr. <i>quadrirubata</i>	676	BC-II
outgroup	Eo00374	<i>Idae</i> <i>quadrirubata</i>	650	Q3-1
single	Eo00003	<i>E.</i> spnr. <i>cancellata</i>	676	Q2#2
single	Eo00013	<i>E.</i> spnr. <i>catana</i>	676	Q3#3
single	Eo00023	<i>E.</i> <i>antiopata</i>	676	Q2#3
single	Eo00024	<i>E.</i> <i>paraviolascens</i>	676	Q2#3
single	Eo00060	<i>E.</i> <i>inflammata</i>	676	Q3#3
single	Eo00082	<i>E.</i> sp.	676	Q3#2
single	Eo00090	<i>E.</i> spnr. <i>olivaria</i>	676	Q2#1
single	Eo00095	<i>E.</i> spnr. 2133	658	Q5#3
single	Eo00099	<i>E.</i> spnr. <i>tegularia</i> 961	676	Q2#3
single	Eo00103	<i>E.</i> sp. 1070	676	Q5#2
single	Eo00107	<i>E.</i> spnr. <i>chasca</i>	676	Q5#3
single	Eo00139	<i>E.</i> spnr. <i>tegularia</i> 961	676	Q2#3
single	Eo00143	<i>E.</i> <i>carrasca</i>	676	Q5#2
single	Eo00144	<i>E.</i> spnr. <i>goodmani</i>	676	Q3#2
single	Eo00166	<i>E.</i> <i>sagittaria</i>	676	Q2#3
single	Eo00169	<i>E.</i> spnr. <i>goodmani</i>	676	Q5#3
single	Eo00181	<i>E.</i> spnr. <i>borrata</i>	676	Q2#3
single	Eo00187	<i>E.</i> spnr. <i>haematodes</i> 977	676	Q5#3
single	Eo00205	<i>E.</i> sp. 420	676	Q3#1
single	Eo00214	<i>E.</i> spnr. <i>sagittaria</i>	676	Q3#3
single	Eo00219	<i>E.</i> spnr. <i>golosata</i>	676	Q3#2
single	Eo00238	<i>E.</i> spnr. <i>concatenata</i>	676	Q5#2
single	Eo00239	<i>E.</i> spnr. <i>pallidicosta</i>	676	Q5#2
single	Eo00245	<i>E.</i> spnr. <i>basaliata</i> 401	676	Q5#3
single	Eo00311	<i>E.</i> sp. 382	676	Q2#3
single	Eo00323	<i>E.</i> sp. 2128	650	Q3#2
single	Eo00326	<i>E.</i> spnr. 2129	676	Q2#3
single	Eo00335	<i>E.</i> spnr. <i>olivacea</i>	676	BC-IV
single	Eo00339	<i>E.</i> sp.	676	BC-I
single	Eo00340	<i>E.</i> <i>nigrostickta</i> 388	676	BC-I
single	Eo00345	<i>E.</i> <i>camptographata</i>	676	BC-II
single	Eo00346	<i>E.</i> spnr. <i>binaria</i>	676	BC-II
single	Eo00349	<i>E.</i> sp.	676	BC-VI
single	Eo00353	<i>E.</i> spnr. <i>paulone</i>	676	4a
single	Eo00381	<i>E.</i> spnr <i>ciocolatina</i>	650	4a
single	Eo00385	<i>E.</i> <i>parva</i>	676	1685
single	Eo00386	<i>E.</i> <i>multistrigaria</i>	676	1686
Sp001	Eo00020	<i>E.</i> <i>ciocolatina</i>	676	Q3#1
Sp001	Eo00022	<i>E.</i> <i>ciocolatina</i>	676	Q2#3
Sp001	Eo00032	<i>E.</i> <i>ciocolatina</i>	676	Q2#2
Sp001	Eo00064	<i>E.</i> <i>ciocolatina</i>	676	Q2#2
Sp001	Eo00079	<i>E.</i> <i>ciocolatina</i>	676	Q2#2
Sp001	Eo00088	<i>E.</i> <i>ciocolatina</i>	676	Q2#3
Sp001	Eo00097	<i>E.</i> <i>ciocolatina</i>	676	Q2#3
Sp001	Eo00100	<i>E.</i> <i>ciocolatina</i>	676	Q2#3
Sp001	Eo00101	<i>E.</i> <i>ciocolatina</i>	650	Q2#3
Sp001	Eo00116	<i>E.</i> <i>ciocolatina</i>	676	Q2#3
Sp001	Eo00142	<i>E.</i> <i>ciocolatina</i>	676	Q5#2
Sp001	Eo00158	<i>E.</i> <i>ciocolatina</i>	676	Q3#2
Sp001	Eo00165	<i>E.</i> <i>ciocolatina</i>	676	Q2#2
Sp001	Eo00168	<i>E.</i> <i>ciocolatina</i>	676	Q5#3
Sp001	Eo00177	<i>E.</i> <i>ciocolatina</i>	676	Q2#3
Sp001	Eo00351	<i>E.</i> <i>ciocolatina</i>	676	4a
Sp001	Eo00366	<i>E.</i> <i>ciocolatina</i>	676	Q3-1
Sp001	Eo00372	<i>E.</i> <i>ciocolatina</i>	676	Q3-1
Sp001	Eo00376	<i>E.</i> <i>ciocolatina</i>	676	Q3-1
Sp002	Eo00034	<i>E.</i> spnr. <i>ciocolatina</i>	676	Q2#2
Sp002	Eo00043	<i>E.</i> spnr. <i>ciocolatina</i>	676	Q2#3
Sp002	Eo00102	<i>E.</i> spnr. <i>ciocolatina</i>	676	Q2#3
Sp002	Eo00153	<i>E.</i> spnr. <i>ciocolatina</i>	676	Q2#3
Sp002	Eo00176	<i>E.</i> spnr. <i>ciocolatina</i>	676	Q2#3
Sp002	Eo00210	<i>E.</i> spnr. <i>ciocolatina</i>	676	Q3#1
Sp002	Eo00373	<i>E.</i> spnr. <i>ciocolatina</i>	676	Q3-1
Sp003	Eo00269	<i>E.</i> sp.	676	Q3#3
Sp003	Eo00273	<i>E.</i> sp.	658	Q3#3
Sp003	Eo00278	<i>E.</i> sp.	676	Q3#3
Sp003	Eo00290	<i>E.</i> sp.	676	Q3#3
Sp003	Eo00316	<i>E.</i> sp.	676	Q3#3
Sp004	Eo00129	<i>E.</i> sp.	676	Q2#3
Sp004	Eo00331	<i>E.</i> sp.	676	Q5#2
Sp005	Eo00173	<i>E.</i> sp. 405	676	Q3#3
Sp005	Eo00261	<i>E.</i> sp. 405	676	Q5#3
Sp006	Eo00062	<i>E.</i> <i>necula</i>	676	Q3#3
Sp006	Eo00125	<i>E.</i> <i>necula</i>	676	Q3#3
Sp007	Eo00008	<i>E.</i> <i>biradiata</i>	676	Q3#3
Sp007	Eo00227	<i>E.</i> <i>biradiata</i>	676	Q3#1
Sp007	Eo00272	<i>E.</i> <i>biradiata</i>	658	Q3#3
Sp007	Eo00286	<i>E.</i> <i>biradiata</i>	676	Q3#3
Sp007	Eo00304	<i>E.</i> <i>biradiata</i>	676	Q3#3
Sp007	Eo00365	<i>E.</i> <i>biradiata</i>	676	Q3-1
Sp009	Eo00180	<i>E.</i> spnr. <i>nigrostickta</i> 388	676	Q2#3

Sp009	Eo00207	<i>E. spnr. nigrosticta</i> 388	676	Q3#1
Sp009	Eo00218	<i>E. spnr. nigrosticta</i> 388	676	Q5#3
Sp010	Eo00163	<i>E. spnr. fucosa</i>	676	Q3#1
Sp010	Eo00186	<i>E. spnr. fucosa</i>	676	Q5#3
Sp010	Eo00197	<i>E. spnr. fucosa</i>	676	Q3#1
Sp010	Eo00315	<i>E. spnr. fucosa</i>	676	Q5#1
Sp010	Eo00319	<i>E. spnr. fucosa</i>	676	Q5#1
Sp010	Eo00324	<i>E. spnr. fucosa</i>	656	Q2#2
Sp010	Eo00325	<i>E. spnr. fucosa</i>	676	Q2#2
Sp011	Eo00255	<i>E. angulata</i>	676	Q5#1
Sp011	Eo00341	<i>E. angulata</i>	676	BC-1
Sp012	Eo00016	<i>E. tegularia</i> 836	676	Q3#1
Sp012	Eo00072	<i>E. tegularia</i> 836	676	Q5#2
Sp012	Eo00109	<i>E. tegularia</i> 836	676	Q2#3
Sp012	Eo00309	<i>E. tegularia</i> 836	676	Q5#1
Sp013	Eo00073	<i>E. spnr. odatis</i> 419	676	Q3#3
Sp013	Eo00114	<i>E. spnr. odatis</i> 419	676	Q2#3
Sp013	Eo00115	<i>E. spnr. odatis</i> 419	676	Q2#3
Sp013	Eo00141	<i>E. spnr. odatis</i> 419	676	Q2#3
Sp013	Eo00265	<i>E. spnr. odatis</i> 419	676	Q5#3
Sp013	Eo00313	<i>E. spnr. odatis</i> 419	635	Q2#3
Sp013	Eo00332	<i>E. spnr. odatis</i> 419	676	Q5#2
Sp014	Eo00226	<i>E. sp.</i>	676	Q3#1
Sp014	Eo00362	<i>E. sp.</i>	676	Q3-1
Sp015	Eo00019	<i>E. sp. 427</i>	676	Q3#1
Sp015	Eo00192	<i>E. sp. 427</i>	676	Q3#1
Sp015	Eo00308	<i>E. sp. 427</i>	647	Q5#2
Sp016	Eo00111	<i>E. trillista</i>	676	Q2#3
Sp016	Eo00112	<i>E. trillista</i>	676	Q2#3
Sp016	Eo00119	<i>E. trillista</i>	676	Q2#3
Sp017	Eo00001	<i>E. lunifera</i>	676	Q2#3
Sp017	Eo00042	<i>E. lunifera</i>	676	Q2#3
Sp017	Eo00120	<i>E. lunifera</i>	676	Q2#3
Sp017	Eo00198	<i>E. lunifera</i>	676	Q3#1
Sp017	Eo00243	<i>E. lunifera</i>	658	Q5#2
Sp017	Eo00256	<i>E. lunifera</i>	676	Q5#3
Sp017	Eo00257	<i>E. lunifera</i>	676	Q5#3
Sp017	Eo00296	<i>E. lunifera</i>	676	Q5#1
Sp018	Eo00025	<i>E. sp. 820</i>	676	Q2#3
Sp018	Eo00048	<i>E. sp. 820</i>	676	Q3#3
Sp018	Eo00131	<i>E. sp. 820</i>	676	Q2#3
Sp018	Eo00155	<i>E. sp. 820</i>	676	Q2#3
Sp018	Eo00295	<i>E. sp. 820</i>	676	Q3#3
Sp018	Eo00317	<i>E. sp. 820</i>	676	Q5#2
Sp019	Eo00004	<i>E. chasca</i>	676	Q3#2
Sp019	Eo00036	<i>E. chasca</i>	676	Q2#1
Sp019	Eo00050	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00096	<i>E. chasca</i>	676	Q5#3
Sp019	Eo00124	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00146	<i>E. chasca</i>	676	Q3#2
Sp019	Eo00161	<i>E. chasca</i>	676	Q3#1
Sp019	Eo00170	<i>E. chasca</i>	661	Q5#3
Sp019	Eo00175	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00178	<i>E. chasca</i>	676	Q2#3
Sp019	Eo00215	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00251	<i>E. chasca</i>	676	Q5#1
Sp019	Eo00258	<i>E. chasca</i>	676	Q5#3
Sp019	Eo00292	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00360	<i>E. chasca</i>	676	Q3-1
Sp019	Eo00368	<i>E. chasca</i>	676	Q3-1
Sp020	Eo00041	<i>E. spnr. sp. 2133</i>	676	Q2#3
Sp020	Eo00069	<i>E. spnr. sp. 2133</i>	676	Q2#2
Sp020	Eo00086	<i>E. spnr. sp. 2133</i>	676	Q2#3
Sp020	Eo00270	<i>E. spnr. sp. 2133</i>	676	Q3#3
Sp021	Eo00098	<i>E. spnr. inflammata</i> 515	676	Q2#3
Sp021	Eo00206	<i>E. spnr. inflammata</i> 515	676	Q3#1
Sp021	Eo00230	<i>E. spnr. inflammata</i> 515	676	Q5#1
Sp022	Eo00149	<i>E. spnr. encina</i>	676	Q3#2
Sp022	Eo00262	<i>E. spnr. encina</i>	676	Q2#1
Sp023	Eo00108	<i>E. spnr. pallidicosta</i>	658	Q5#3
Sp023	Eo00217	<i>E. spnr. pallidicosta</i>	676	Q5#3
Sp024	Eo00110	<i>E. pallidicosta</i>	676	Q2#3
Sp024	Eo00138	<i>E. pallidicosta</i>	676	Q2#1
Sp024	Eo00242	<i>E. pallidicosta</i>	676	Q5#2
Sp024	Eo00244	<i>E. pallidicosta</i>	676	Q5#3
Sp024	Eo00329	<i>E. pallidicosta</i>	676	Q3#3
Sp025	Eo00045	<i>E. muscosa</i>	676	Q2#3
Sp025	Eo00136	<i>E. muscosa</i>	676	Q2#2
Sp025	Eo00140	<i>E. muscosa</i>	676	Q2#3
Sp025	Eo00162	<i>E. muscosa</i>	676	Q3#1
Sp025	Eo00204	<i>E. muscosa</i>	676	Q3#1
Sp025	Eo00254	<i>E. muscosa</i>	676	Q5#1
Sp026	Eo00123	<i>E. spnr. goodmani</i>	676	Q5#3
Sp026	Eo00132	<i>E. spnr. goodmani</i>	676	Q2#3

Sp026	Eo00133	<i>E. spnr. goodmani</i>	676	Q2#3
Sp026	Eo00185	<i>E. spnr. goodmani</i>	676	Q5#3
Sp026	Eo00246	<i>E. spnr. goodmani</i>	676	Q5#3
Sp026	Eo00298	<i>E. spnr. goodmani</i>	650	Q5#1
Sp026	Eo00300	<i>E. spnr. goodmani</i>	676	Q5#1
Sp026	Eo00322	<i>E. spnr. goodmani</i>	676	Q3#2
Sp027	Eo00044	<i>E. spnr. goodmani</i>	676	Q2#3
Sp027	Eo00074	<i>E. spnr. goodmani</i>	676	Q3#3
Sp027	Eo00179	<i>E. spnr. goodmani</i>	676	Q2#3
Sp027	Eo00183	<i>E. spnr. goodmani</i>	676	Q5#3
Sp028	Eo00014	<i>E. spnr. goodmani</i>	676	Q3#3
Sp028	Eo00274	<i>E. spnr. goodmani</i>	676	Q3#3
Sp028	Eo00289	<i>E. spnr. goodmani</i>	676	Q3#3
Sp028	Eo00294	<i>E. spnr. goodmani</i>	676	Q3#3
Sp029	Eo00209	<i>E. spnr. goodmani</i>	676	Q3#1
Sp029	Eo00228	<i>E. spnr. goodmani</i>	676	Q3#1
Sp029	Eo00231	<i>E. spnr. goodmani</i>	676	Q5#1
Sp029	Eo00248	<i>E. spnr. goodmani</i>	676	Q5#1
Sp030	Eo00263	<i>E. spnr. olivacea</i>	676	Q2#3
Sp030	Eo00356	<i>E. spnr. olivacea</i>	676	4a
Sp031	Eo00047	<i>E. spnr. olivacea</i>	676	Q3#3
Sp031	Eo00087	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00089	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00182	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00213	<i>E. spnr. olivacea</i>	676	Q3#1
Sp031	Eo00223	<i>E. spnr. olivacea</i>	676	Q3#1
Sp031	Eo00260	<i>E. spnr. olivacea</i>	676	Q5#3
Sp031	Eo00264	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00363	<i>E. spnr. olivacea</i>	676	Q3-1
Sp032	Eo00031	<i>E. spnr. olivacea</i>	676	Q2#1
Sp032	Eo00085	<i>E. spnr. olivacea</i>	676	Q2#3
Sp032	Eo00105	<i>E. spnr. olivacea</i>	676	Q5#2
Sp032	Eo00106	<i>E. spnr. olivacea</i>	676	Q5#2
Sp032	Eo00113	<i>E. spnr. olivacea</i>	676	Q2#3
Sp032	Eo00117	<i>E. spnr. olivacea</i>	676	Q2#3
Sp032	Eo00127	<i>E. spnr. olivacea</i>	676	Q2#3
Sp032	Eo00137	<i>E. spnr. olivacea</i>	676	Q2#1
Sp032	Eo00171	<i>E. spnr. olivacea</i>	676	Q5#3
Sp032	Eo00200	<i>E. spnr. olivacea</i>	676	Q5#3
Sp032	Eo00241	<i>E. spnr. olivacea</i>	676	Q5#2
Sp032	Eo00252	<i>E. spnr. olivacea</i>	676	Q5#1
Sp032	Eo00301	<i>E. spnr. olivacea</i>	676	Q5#1
Sp032	Eo00303	<i>E. spnr. olivacea</i>	676	Q5#1
Sp033	Eo00030	<i>E. chrysocraspedata</i>	676	Q2#1
Sp033	Eo00033	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00038	<i>E. chrysocraspedata</i>	676	Q2#1
Sp033	Eo00067	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00068	<i>E. chrysocraspedata</i>	650	Q2#2
Sp033	Eo00077	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00078	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00145	<i>E. chrysocraspedata</i>	676	Q3#2
Sp033	Eo00147	<i>E. chrysocraspedata</i>	647	Q3#2
Sp033	Eo00148	<i>E. chrysocraspedata</i>	676	Q3#2
Sp033	Eo00160	<i>E. chrysocraspedata</i>	676	Q3#2
Sp033	Eo00164	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00196	<i>E. chrysocraspedata</i>	676	Q3#1
Sp033	Eo00201	<i>E. chrysocraspedata</i>	676	Q5#3
Sp033	Eo00202	<i>E. chrysocraspedata</i>	653	Q5#3
Sp033	Eo00208	<i>E. chrysocraspedata</i>	676	Q3#1
Sp033	Eo00221	<i>E. chrysocraspedata</i>	676	Q3#1
Sp033	Eo00222	<i>E. chrysocraspedata</i>	676	Q2#1
Sp033	Eo00229	<i>E. chrysocraspedata</i>	676	Q3#1
Sp033	Eo00247	<i>E. chrysocraspedata</i>	635	Q5#1
Sp033	Eo00250	<i>E. chrysocraspedata</i>	676	Q5#1
Sp033	Eo00361	<i>E. chrysocraspedata</i>	676	Q3-1
Sp033	Eo00369	<i>E. chrysocraspedata</i>	676	Q3-1
Sp033	Eo00377	<i>E. chrysocraspedata</i>	676	Q3-1
Sp034	Eo00017	<i>E. plana</i>	663	Q3#1
Sp034	Eo00029	<i>E. plana</i>	676	Q3#2
Sp034	Eo00039	<i>E. plana</i>	676	Q2#1
Sp034	Eo00040	<i>E. plana</i>	676	Q3#1
Sp034	Eo00081	<i>E. plana</i>	644	Q3#2
Sp034	Eo00083	<i>E. plana</i>	676	Q3#2
Sp034	Eo00091	<i>E. plana</i>	676	Q2#1
Sp034	Eo00094	<i>E. plana</i>	676	Q5#3
Sp034	Eo00193	<i>E. plana</i>	676	Q3#1
Sp034	Eo00195	<i>E. plana</i>	676	Q3#1
Sp034	Eo00203	<i>E. plana</i>	676	Q5#3
Sp034	Eo00225	<i>E. plana</i>	676	Q3#1
Sp035	Eo00065	<i>E. sp.</i>	676	Q2#2
Sp035	Eo00070	<i>E. sp.</i>	676	Q5#2
Sp035	Eo00184	<i>E. sp.</i>	676	Q5#3
Sp035	Eo00194	<i>E. sp.</i>	676	Q3#1
Sp035	Eo00224	<i>E. sp.</i>	676	Q3#1

Sp035	Eo00364	<i>E. sp.</i>	676	Q3-1
Sp036	Eo00027	<i>E. xanthoperata</i>	650	Q3#2
Sp036	Eo00066	<i>E. xanthoperata</i>	676	Q2#2
Sp037	Eo00018	<i>E. dorisaria</i>	651	Q3#1
Sp037	Eo00190	<i>E. dorisaria</i>	676	Q2#1
Sp037	Eo00191	<i>E. dorisaria</i>	676	Q2#1
Sp037	Eo00232	<i>E. dorisaria</i>	676	Q5#1
Sp037	Eo00233	<i>E. dorisaria</i>	676	Q5#1
Sp037	Eo00237	<i>E. dorisaria</i>	676	Q5#1
Sp037	Eo00253	<i>E. dorisaria</i>	676	Q5#1
Sp037	Eo00358	<i>E. dorisaria</i>	676	Q3-1
Sp037	Eo00359	<i>E. dorisaria</i>	676	Q3-1
Sp037	Eo00371	<i>E. dorisaria</i>	676	Q3-1
Sp037	Eo00375	<i>E. dorisaria</i>	676	Q3-1
Sp039	Eo00338	<i>E. catana</i>	676	BC-1
Sp039	Eo00352	<i>E. catana</i>	676	4a
Sp040	Eo00005	<i>E. planetaria</i>	676	Q3#3
Sp040	Eo00093	<i>E. planetaria</i>	676	Q2#3
Sp040	Eo00118	<i>E. planetaria</i>	676	Q3#3
Sp040	Eo00126	<i>E. planetaria</i>	676	Q3#3
Sp040	Eo00150	<i>E. planetaria</i>	676	Q3#2
Sp040	Eo00152	<i>E. planetaria</i>	676	Q2#3
Sp040	Eo00299	<i>E. planetaria</i>	676	Q5#1
Sp041	Eo00283	<i>E. lucivittata</i> 384	676	Q3#3
Sp041	Eo00379	<i>E. lucivittata</i> 384	676	4a
Sp041	Eo00380	<i>E. lucivittata</i> 384	651	4a
Sp042	Eo00154	<i>E. spnr. azafranata</i>	658	Q2#3
Sp042	Eo00167	<i>E. spnr. azafranata</i>	650	Q5#3
Sp042	Eo00199	<i>E. spnr. azafranata</i>	676	Q5#3
Sp042	Eo00266	<i>E. spnr. azafranata</i>	635	Q5#3
Sp042	Eo00281	<i>E. spnr. azafranata</i>	676	Q3#3
Sp042	Eo00291	<i>E. spnr. azafranata</i>	676	Q3#3
Sp042	Eo00310	<i>E. spnr. azafranata</i>	676	Q2#3
Sp042	Eo00312	<i>E. spnr. azafranata</i>	676	Q2#3
Sp044	Eo00026	<i>E. escamata</i>	676	Q2#3
Sp044	Eo00035	<i>E. escamata</i>	676	Q2#2
Sp045	Eo00006	<i>E. burla</i>	676	Q3#3
Sp045	Eo00061	<i>E. burla</i>	650	Q3#3
Sp045	Eo00080	<i>E. burla</i>	676	Q2#2
Sp045	Eo00084	<i>E. burla</i>	674	Q2#3
Sp045	Eo00121	<i>E. burla</i>	676	Q5#3
Sp045	Eo00156	<i>E. burla</i>	664	Q2#3
Sp045	Eo00343	<i>E. burla</i>	676	KP1-VI
Sp046	Eo00188	<i>E. spnr. adimaria</i> 399	676	Q5#3
Sp047	Eo00189	<i>E. spnr. borrata</i>	676	Q5#3
Sp046	Eo00287	<i>E. spnr. adimaria</i> 399	676	Q3#3
Sp047	Eo00333	<i>E. spnr. borrata</i>	676	Q5#2
Sp047	Eo00388	<i>E. spnr. borrata</i>	676	Q5#1
Sp048	Eo00348	<i>E. spnr. yvatta</i> 400	676	BC-VI
Sp048	Eo00350	<i>E. spnr. yvatta</i> 400	676	BC-VI
Sp049	Eo00012	<i>E. spnr. golosata</i>	676	Q3#3
Sp049	Eo00157	<i>E. spnr. golosata</i>	676	Q2#3
Sp049	Eo00159	<i>E. spnr. golosata</i>	658	Q3#2
Sp049	Eo00240	<i>E. spnr. golosata</i>	676	Q5#2
Sp049	Eo00271	<i>E. spnr. golosata</i>	658	Q3#3
Sp049	Eo00321	<i>E. spnr. golosata</i>	676	Q3#2
Sp049	Eo00330	<i>E. spnr. golosata</i>	676	Q3#3
Sp050	Eo00028	<i>E. spnr. golosata</i>	676	Q3#2
Sp050	Eo00288	<i>E. spnr. golosata</i>	676	Q3#3
Sp050	Eo00305	<i>E. spnr. golosata</i>	676	Q3#3
Sp050	Eo00307	<i>E. spnr. golosata</i>	676	Q5#2
Sp051	Eo00010	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00037	<i>E. spnr. margarita</i>	665	Q2#1
Sp051	Eo00302	<i>E. spnr. margarita</i>	676	Q5#1
Sp051	Eo00378	<i>E. spnr. margarita</i>	676	4a
Sp052	Eo00002	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00007	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00009	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00011	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00049	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00051	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00075	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00076	<i>E. spnr. margarita</i>	652	Q3#3
Sp052	Eo00151	<i>E. spnr. margarita</i>	676	Q2#3
Sp052	Eo00172	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00212	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00220	<i>E. spnr. margarita</i>	676	Q3#2
Sp052	Eo00236	<i>E. spnr. margarita</i>	658	Q5#1
Sp052	Eo00268	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00285	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00355	<i>E. spnr. margarita</i>	676	4a
Sp053	Eo00021	<i>E. sp. 409</i>	676	Q3#1
Sp053	Eo00063	<i>E. sp. 409</i>	676	Q2#2
Sp053	Eo00128	<i>E. sp. 409</i>	676	Q2#3



Sp054	Eo00071	<i>E. spnr. borrata</i>	636	Q5#2
Sp054	Eo00092	<i>E. spnr. borrata</i>	676	Q2#3
Sp054	Eo00130	<i>E. spnr. borrata</i>	635	Q2#3
Sp054	Eo00282	<i>E. spnr. borrata</i>	658	Q3#3
Sp054	Eo00284	<i>E. spnr. borrata</i>	658	Q3#3
Sp054	Eo00327	<i>E. spnr. borrata</i>	635	Q2#3
unknown	Eo00342		635	BC-I

**Table A5.** 2% threshold delimitation: List of all individuals with their species assignment, length of barcoding sequence and collection site indicated. Numeric species codes are given for reference to G. Brehm's database where applicable.

Species	Code	Name	Length	Collection site
outgroup	Eo00052	<i>Lissochlora diarita</i>	676	Q3#1
outgroup	Eo00053	<i>Cargolia</i> sp.	676	Q3#1
outgroup	Eo00054	<i>Chloropteryx opalaria</i>	676	Q3#1
outgroup	Eo00055		676	Q3#1
outgroup	Eo00056	<i>Cargolia arana</i>	676	Q3#1
outgroup	Eo00057	<i>Cargolia arana</i>	676	Q3#1
outgroup	Eo00058	<i>Cargolia arana</i>	676	Q3#1
outgroup	Eo00059		676	Q3#1
outgroup	Eo00104	<i>Cyclophora dorsivena</i>	676	Q5#2
outgroup	Eo00134	<i>Dithecodes distracta</i>	676	Q2#3
outgroup	Eo00135	<i>Chloropteryx punctilinea</i>	676	Q2#3
outgroup	Eo00211	<i>Idaea quadrirubata</i>	676	Q3#3
outgroup	Eo00259	<i>Idaea recrinita</i>	637	Q5#3
outgroup	Eo00334	<i>Cyclophora</i> sp.	676	BC-IV
outgroup	Eo00344	<i>Lobocleta costalis</i>	676	BC-II
outgroup	Eo00347	<i>Idaea</i> spnr. <i>quadrirubata</i>	676	BC-II
outgroup	Eo00374	<i>Idaea quadrirubata</i>	650	Q3-1
single	Eo00003	<i>E. spnr. cancellata</i>	676	Q2#2
single	Eo00013	<i>E. spnr. catana</i>	676	Q3#3
single	Eo00023	<i>E. antiopata</i>	676	Q2#3
single	Eo00024	<i>E. paraviolascens</i>	676	Q2#3
single	Eo00060	<i>E. inflammata</i>	676	Q3#3
single	Eo00082	<i>E. sp.</i>	676	Q3#2
single	Eo00090	<i>E. spnr. olivaria</i>	676	Q2#1
single	Eo00095	<i>E. spnr. 2133</i>	658	Q5#3
single	Eo00099	<i>E. spnr. tegularia 961</i>	676	Q2#3
single	Eo00103	<i>E. sp. 1070</i>	676	Q5#2
single	Eo00107	<i>E. spnr. chasca</i>	676	Q5#3
single	Eo00139	<i>E. spnr. tegularia 961</i>	676	Q2#3
single	Eo00143	<i>E. carrasca</i>	676	Q5#2
single	Eo00144	<i>E. spnr. goodmani</i>	676	Q3#2
single	Eo00166	<i>E. sagittaria</i>	676	Q2#3
single	Eo00169	<i>E. spnr. goodmani</i>	676	Q5#3
single	Eo00181	<i>E. spnr. borrata</i>	676	Q2#3
single	Eo00187	<i>E. spnr. haematodes 977</i>	676	Q5#3
single	Eo00205	<i>E. sp. 420</i>	676	Q3#1
single	Eo00214	<i>E. spnr. sagittaria</i>	676	Q3#3
single	Eo00218	<i>E. spnr. nigrosticta 388</i>	676	Q5#3
single	Eo00219	<i>E. spnr. golosata</i>	676	Q3#2
single	Eo00238	<i>E. spnr. concatenata</i>	676	Q5#2
single	Eo00239	<i>E. spnr. pallidicosta</i>	676	Q5#2
single	Eo00245	<i>E. spnr. basaliata 401</i>	676	Q5#3
single	Eo00311	<i>E. sp. 382</i>	676	Q2#3
single	Eo00323	<i>E. sp. 2128</i>	650	Q3#2
single	Eo00326	<i>E. spnr. 2129</i>	676	Q2#3
single	Eo00335	<i>E. spnr. olivacea</i>	676	BC-IV
single	Eo00339	<i>E. sp.</i>	676	BC-I
single	Eo00340	<i>E. nigrosticta 388</i>	676	BC-I
single	Eo00345	<i>E. camptographata</i>	676	BC-II
single	Eo00346	<i>E. spnr. binaria</i>	676	BC-II
single	Eo00349	<i>E. sp.</i>	676	BC-VI
single	Eo00353	<i>E. spnr. paulone</i>	676	4a
single	Eo00381	<i>E. spnr. ciocolatina</i>	650	4a
single	Eo00385	<i>E. parva</i>	676	1685
single	Eo00386	<i>E. multistrigaria</i>	676	1686
Sp001	Eo00020	<i>E. ciocolatina</i>	676	Q3#1
Sp001	Eo00022	<i>E. ciocolatina</i>	676	Q2#3
Sp001	Eo00032	<i>E. ciocolatina</i>	676	Q2#2
Sp001	Eo00064	<i>E. ciocolatina</i>	676	Q2#2
Sp001	Eo00079	<i>E. ciocolatina</i>	676	Q2#2
Sp001	Eo00088	<i>E. ciocolatina</i>	676	Q2#3
Sp001	Eo00097	<i>E. ciocolatina</i>	676	Q2#3
Sp001	Eo00100	<i>E. ciocolatina</i>	676	Q2#3
Sp001	Eo00101	<i>E. ciocolatina</i>	650	Q2#3
Sp001	Eo00116	<i>E. ciocolatina</i>	676	Q2#3
Sp001	Eo00142	<i>E. ciocolatina</i>	676	Q5#2
Sp001	Eo00158	<i>E. ciocolatina</i>	676	Q3#2
Sp001	Eo00165	<i>E. ciocolatina</i>	676	Q2#2
Sp001	Eo00168	<i>E. ciocolatina</i>	676	Q5#3

Sp001	Eo00177	<i>E. ciocolatina</i>	676	Q2#3
Sp001	Eo00351	<i>E. ciocolatina</i>	676	4a
Sp001	Eo00366	<i>E. ciocolatina</i>	676	Q3-1
Sp001	Eo00372	<i>E. ciocolatina</i>	676	Q3-1
Sp001	Eo00376	<i>E. ciocolatina</i>	676	Q3-1
Sp002	Eo00034	<i>E. spnr. ciocolatina</i>	676	Q2#2
Sp002	Eo00043	<i>E. spnr. ciocolatina</i>	676	Q2#3
Sp002	Eo00102	<i>E. spnr. ciocolatina</i>	676	Q2#3
Sp002	Eo00153	<i>E. spnr. ciocolatina</i>	676	Q2#3
Sp002	Eo00176	<i>E. spnr. ciocolatina</i>	676	Q2#3
Sp002	Eo00210	<i>E. spnr. ciocolatina</i>	676	Q3#1
Sp002	Eo00373	<i>E. spnr. ciocolatina</i>	676	Q3-1
Sp003	Eo00269	<i>E. sp.</i>	676	Q3#3
Sp003	Eo00273	<i>E. sp.</i>	658	Q3#3
Sp003	Eo00278	<i>E. sp.</i>	676	Q3#3
Sp003	Eo00290	<i>E. sp.</i>	676	Q3#3
Sp003	Eo00316	<i>E. sp.</i>	676	Q3#3
Sp004	Eo00129	<i>E. sp.</i>	676	Q2#3
Sp004	Eo00331	<i>E. sp.</i>	676	Q5#2
Sp005	Eo00173	<i>E. sp. 405</i>	676	Q3#3
Sp005	Eo00261	<i>E. sp. 405</i>	676	Q5#3
Sp006	Eo00062	<i>E. necula</i>	676	Q3#3
Sp006	Eo00125	<i>E. necula</i>	676	Q3#3
Sp007	Eo00008	<i>E. biradiata</i>	676	Q3#3
Sp007	Eo00272	<i>E. biradiata</i>	658	Q3#3
Sp007	Eo00286	<i>E. biradiata</i>	676	Q3#3
Sp007	Eo00304	<i>E. biradiata</i>	676	Q3#3
Sp008	Eo00227	<i>E. biradiata</i>	676	Q3#1
Sp008	Eo00365	<i>E. biradiata</i>	676	Q3-1
Sp009	Eo00180	<i>E. spnr. nigrosticta 388</i>	676	Q2#3
Sp009	Eo00207	<i>E. spnr. nigrosticta 388</i>	676	Q3#1
Sp010	Eo00163	<i>E. spnr. fucosa</i>	676	Q3#1
Sp010	Eo00186	<i>E. spnr. fucosa</i>	676	Q5#3
Sp010	Eo00197	<i>E. spnr. fucosa</i>	676	Q3#1
Sp010	Eo00315	<i>E. spnr. fucosa</i>	676	Q5#1
Sp010	Eo00319	<i>E. spnr. fucosa</i>	676	Q5#1
Sp010	Eo00324	<i>E. spnr. fucosa</i>	656	Q2#2
Sp010	Eo00325	<i>E. spnr. fucosa</i>	676	Q2#2
Sp011	Eo00255	<i>E. angulata</i>	676	Q5#1
Sp011	Eo00341	<i>E. angulata</i>	676	BC-1
Sp012	Eo00016	<i>E. tegularia 836</i>	676	Q3#1
Sp012	Eo00072	<i>E. tegularia 836</i>	676	Q5#2
Sp012	Eo00109	<i>E. tegularia 836</i>	676	Q2#3
Sp012	Eo00309	<i>E. tegularia 836</i>	676	Q5#1
Sp013	Eo00073	<i>E. spnr. odatis 419</i>	676	Q3#3
Sp013	Eo00114	<i>E. spnr. odatis 419</i>	676	Q2#3
Sp013	Eo00115	<i>E. spnr. odatis 419</i>	676	Q2#3
Sp013	Eo00141	<i>E. spnr. odatis 419</i>	676	Q2#3
Sp013	Eo00265	<i>E. spnr. odatis 419</i>	676	Q5#3
Sp013	Eo00313	<i>E. spnr. odatis 419</i>	635	Q2#3
Sp013	Eo00332	<i>E. spnr. odatis 419</i>	676	Q5#2
Sp014	Eo00226	<i>E. sp.</i>	676	Q3#1
Sp014	Eo00362	<i>E. sp.</i>	676	Q3-1
Sp015	Eo00019	<i>E. sp. 427</i>	676	Q3#1
Sp015	Eo00192	<i>E. sp. 427</i>	676	Q3#1
Sp015	Eo00308	<i>E. sp. 427</i>	647	Q5#2
Sp016	Eo00111	<i>E. trillista</i>	676	Q2#3
Sp016	Eo00112	<i>E. trillista</i>	676	Q2#3
Sp016	Eo00119	<i>E. trillista</i>	676	Q2#3
Sp017	Eo00001	<i>E. lunifera</i>	676	Q2#3
Sp017	Eo00042	<i>E. lunifera</i>	676	Q2#3
Sp017	Eo00120	<i>E. lunifera</i>	676	Q2#3
Sp017	Eo00198	<i>E. lunifera</i>	676	Q3#1
Sp017	Eo00243	<i>E. lunifera</i>	658	Q5#2
Sp017	Eo00256	<i>E. lunifera</i>	676	Q5#3
Sp017	Eo00257	<i>E. lunifera</i>	676	Q5#3
Sp017	Eo00296	<i>E. lunifera</i>	676	Q5#1
Sp018	Eo00025	<i>E. sp. 820</i>	676	Q2#3
Sp018	Eo00048	<i>E. sp. 820</i>	676	Q3#3
Sp018	Eo00131	<i>E. sp. 820</i>	676	Q2#3
Sp018	Eo00155	<i>E. sp. 820</i>	676	Q2#3
Sp018	Eo00295	<i>E. sp. 820</i>	676	Q3#3
Sp018	Eo00317	<i>E. sp. 820</i>	676	Q5#2
Sp019	Eo00004	<i>E. chasca</i>	676	Q3#2
Sp019	Eo00036	<i>E. chasca</i>	676	Q2#1
Sp019	Eo00050	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00096	<i>E. chasca</i>	676	Q5#3
Sp019	Eo00124	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00146	<i>E. chasca</i>	676	Q3#2
Sp019	Eo00161	<i>E. chasca</i>	676	Q3#1
Sp019	Eo00170	<i>E. chasca</i>	661	Q5#3
Sp019	Eo00175	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00178	<i>E. chasca</i>	676	Q2#3
Sp019	Eo00215	<i>E. chasca</i>	676	Q3#3

Sp019	Eo00251	<i>E. chasca</i>	676	Q5#1
Sp019	Eo00258	<i>E. chasca</i>	676	Q5#3
Sp019	Eo00292	<i>E. chasca</i>	676	Q3#3
Sp019	Eo00360	<i>E. chasca</i>	676	Q3-1
Sp019	Eo00368	<i>E. chasca</i>	676	Q3-1
Sp020	Eo00041	<i>E. spnr. sp. 2133</i>	676	Q2#3
Sp020	Eo00069	<i>E. spnr. sp. 2133</i>	676	Q2#2
Sp020	Eo00086	<i>E. spnr. sp. 2133</i>	676	Q2#3
Sp020	Eo00270	<i>E. spnr. sp. 2133</i>	676	Q3#3
Sp021	Eo00098	<i>E. spnr. inflammata 515</i>	676	Q2#3
Sp021	Eo00206	<i>E. spnr. inflammata 515</i>	676	Q3#1
Sp021	Eo00230	<i>E. spnr. inflammata 515</i>	676	Q5#1
Sp022	Eo00149	<i>E. spnr. encina</i>	676	Q3#2
Sp022	Eo00262	<i>E. spnr. encina</i>	676	Q2#1
Sp023	Eo00108	<i>E. spnr. pallidicosta</i>	658	Q5#3
Sp023	Eo00217	<i>E. spnr. pallidicosta</i>	676	Q5#3
Sp024	Eo00110	<i>E. pallidicosta</i>	676	Q2#3
Sp024	Eo00138	<i>E. pallidicosta</i>	676	Q2#1
Sp024	Eo00242	<i>E. pallidicosta</i>	676	Q5#2
Sp024	Eo00244	<i>E. pallidicosta</i>	676	Q5#3
Sp024	Eo00329	<i>E. pallidicosta</i>	676	Q3#3
Sp025	Eo00045	<i>E. muscosa</i>	676	Q2#3
Sp025	Eo00136	<i>E. muscosa</i>	676	Q2#2
Sp025	Eo00140	<i>E. muscosa</i>	676	Q2#3
Sp025	Eo00162	<i>E. muscosa</i>	676	Q3#1
Sp025	Eo00204	<i>E. muscosa</i>	676	Q3#1
Sp025	Eo00254	<i>E. muscosa</i>	676	Q5#1
Sp026	Eo00123	<i>E. spnr. goodmani</i>	676	Q5#3
Sp026	Eo00132	<i>E. spnr. goodmani</i>	676	Q2#3
Sp026	Eo00133	<i>E. spnr. goodmani</i>	676	Q2#3
Sp026	Eo00185	<i>E. spnr. goodmani</i>	676	Q5#3
Sp026	Eo00246	<i>E. spnr. goodmani</i>	676	Q5#3
Sp026	Eo00298	<i>E. spnr. goodmani</i>	650	Q5#1
Sp026	Eo00300	<i>E. spnr. goodmani</i>	676	Q5#1
Sp026	Eo00322	<i>E. spnr. goodmani</i>	676	Q3#2
Sp027	Eo00044	<i>E. spnr. goodmani</i>	676	Q2#3
Sp027	Eo00074	<i>E. spnr. goodmani</i>	676	Q3#3
Sp027	Eo00179	<i>E. spnr. goodmani</i>	676	Q2#3
Sp027	Eo00183	<i>E. spnr. goodmani</i>	676	Q5#3
Sp028	Eo00014	<i>E. spnr. goodmani</i>	676	Q3#3
Sp028	Eo00274	<i>E. spnr. goodmani</i>	676	Q3#3
Sp028	Eo00289	<i>E. spnr. goodmani</i>	676	Q3#3
Sp028	Eo00294	<i>E. spnr. goodmani</i>	676	Q3#3
Sp029	Eo00209	<i>E. spnr. goodmani</i>	676	Q3#1
Sp029	Eo00228	<i>E. spnr. goodmani</i>	676	Q3#1
Sp029	Eo00231	<i>E. spnr. goodmani</i>	676	Q5#1
Sp029	Eo00248	<i>E. spnr. goodmani</i>	676	Q5#1
Sp030	Eo00263	<i>E. spnr. olivacea</i>	676	Q2#3
Sp030	Eo00356	<i>E. spnr. olivacea</i>	676	4a
Sp031	Eo00047	<i>E. spnr. olivacea</i>	676	Q3#3
Sp031	Eo00087	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00089	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00182	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00213	<i>E. spnr. olivacea</i>	676	Q3#1
Sp031	Eo00223	<i>E. spnr. olivacea</i>	676	Q3#1
Sp031	Eo00260	<i>E. spnr. olivacea</i>	676	Q5#3
Sp031	Eo00264	<i>E. spnr. olivacea</i>	676	Q2#3
Sp031	Eo00363	<i>E. spnr. olivacea</i>	676	Q3-1
Sp032	Eo00031	<i>E. spnr. olivacea</i>	676	Q2#1
Sp032	Eo00085	<i>E. spnr. olivacea</i>	676	Q2#3
Sp032	Eo00105	<i>E. spnr. olivacea</i>	676	Q5#2
Sp032	Eo00106	<i>E. spnr. olivacea</i>	676	Q5#2
Sp032	Eo00113	<i>E. spnr. olivacea</i>	676	Q2#3
Sp032	Eo00117	<i>E. spnr. olivacea</i>	676	Q2#3
Sp032	Eo00127	<i>E. spnr. olivacea</i>	676	Q2#3
Sp032	Eo00137	<i>E. spnr. olivacea</i>	676	Q2#1
Sp032	Eo00171	<i>E. spnr. olivacea</i>	676	Q5#3
Sp032	Eo00200	<i>E. spnr. olivacea</i>	676	Q5#3
Sp032	Eo00241	<i>E. spnr. olivacea</i>	676	Q5#2
Sp032	Eo00252	<i>E. spnr. olivacea</i>	676	Q5#1
Sp032	Eo00301	<i>E. spnr. olivacea</i>	676	Q5#1
Sp032	Eo00303	<i>E. spnr. olivacea</i>	676	Q5#1
Sp033	Eo00030	<i>E. chrysocraspedata</i>	676	Q2#1
Sp033	Eo00033	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00038	<i>E. chrysocraspedata</i>	676	Q2#1
Sp033	Eo00067	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00068	<i>E. chrysocraspedata</i>	650	Q2#2
Sp033	Eo00077	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00078	<i>E. chrysocraspedata</i>	676	Q2#2
Sp033	Eo00145	<i>E. chrysocraspedata</i>	676	Q3#2
Sp033	Eo00147	<i>E. chrysocraspedata</i>	647	Q3#2
Sp033	Eo00148	<i>E. chrysocraspedata</i>	676	Q3#2
Sp033	Eo00160	<i>E. chrysocraspedata</i>	676	Q3#2
Sp033	Eo00164	<i>E. chrysocraspedata</i>	676	Q2#2

Sp033	Eo00196	<i>E. chrysocraspedata</i>	676	Q3#1
Sp033	Eo00201	<i>E. chrysocraspedata</i>	676	Q5#3
Sp033	Eo00202	<i>E. chrysocraspedata</i>	653	Q5#3
Sp033	Eo00208	<i>E. chrysocraspedata</i>	676	Q3#1
Sp033	Eo00221	<i>E. chrysocraspedata</i>	676	Q3#1
Sp033	Eo00222	<i>E. chrysocraspedata</i>	676	Q2#1
Sp033	Eo00229	<i>E. chrysocraspedata</i>	676	Q3#1
Sp033	Eo00247	<i>E. chrysocraspedata</i>	635	Q5#1
Sp033	Eo00250	<i>E. chrysocraspedata</i>	676	Q5#1
Sp033	Eo00361	<i>E. chrysocraspedata</i>	676	Q3-1
Sp033	Eo00369	<i>E. chrysocraspedata</i>	676	Q3-1
Sp033	Eo00377	<i>E. chrysocraspedata</i>	676	Q3-1
Sp034	Eo00017	<i>E. plana</i>	663	Q3#1
Sp034	Eo00029	<i>E. plana</i>	676	Q3#2
Sp034	Eo00039	<i>E. plana</i>	676	Q2#1
Sp034	Eo00040	<i>E. plana</i>	676	Q3#1
Sp034	Eo00081	<i>E. plana</i>	644	Q3#2
Sp034	Eo00083	<i>E. plana</i>	676	Q3#2
Sp034	Eo00091	<i>E. plana</i>	676	Q2#1
Sp034	Eo00094	<i>E. plana</i>	676	Q5#3
Sp034	Eo00193	<i>E. plana</i>	676	Q3#1
Sp034	Eo00195	<i>E. plana</i>	676	Q3#1
Sp034	Eo00203	<i>E. plana</i>	676	Q5#3
Sp034	Eo00225	<i>E. plana</i>	676	Q3#1
Sp035	Eo00065	<i>E. sp.</i>	676	Q2#2
Sp035	Eo00070	<i>E. sp.</i>	676	Q5#2
Sp035	Eo00184	<i>E. sp.</i>	676	Q5#3
Sp035	Eo00194	<i>E. sp.</i>	676	Q3#1
Sp035	Eo00224	<i>E. sp.</i>	676	Q3#1
Sp035	Eo00364	<i>E. sp.</i>	676	Q3-1
Sp036	Eo00027	<i>E. xanthoperata</i>	650	Q3#2
Sp036	Eo00066	<i>E. xanthoperata</i>	676	Q2#2
Sp037	Eo00018	<i>E. dorisaria</i>	651	Q3#1
Sp037	Eo00190	<i>E. dorisaria</i>	676	Q2#1
Sp037	Eo00191	<i>E. dorisaria</i>	676	Q2#1
Sp037	Eo00232	<i>E. dorisaria</i>	676	Q5#1
Sp037	Eo00233	<i>E. dorisaria</i>	676	Q5#1
Sp037	Eo00237	<i>E. dorisaria</i>	676	Q5#1
Sp037	Eo00253	<i>E. dorisaria</i>	676	Q5#1
Sp037	Eo00358	<i>E. dorisaria</i>	676	Q3-1
Sp037	Eo00359	<i>E. dorisaria</i>	676	Q3-1
Sp037	Eo00371	<i>E. dorisaria</i>	676	Q3-1
Sp037	Eo00375	<i>E. dorisaria</i>	676	Q3-1
Sp039	Eo00338	<i>E. catana</i>	676	BC-1
Sp039	Eo00352	<i>E. catana</i>	676	4a
Sp040	Eo00005	<i>E. planetaria</i>	676	Q3#3
Sp040	Eo00093	<i>E. planetaria</i>	676	Q2#3
Sp040	Eo00118	<i>E. planetaria</i>	676	Q3#3
Sp040	Eo00126	<i>E. planetaria</i>	676	Q3#3
Sp040	Eo00150	<i>E. planetaria</i>	676	Q3#2
Sp040	Eo00152	<i>E. planetaria</i>	676	Q2#3
Sp040	Eo00299	<i>E. planetaria</i>	676	Q5#1
Sp041	Eo00283	<i>E. lucivittata</i> 384	676	Q3#3
Sp041	Eo00379	<i>E. lucivittata</i> 384	676	4a
Sp041	Eo00380	<i>E. lucivittata</i> 384	651	4a
Sp042	Eo00154	<i>E. spnr. azafranata</i>	658	Q2#3
Sp042	Eo00167	<i>E. spnr. azafranata</i>	650	Q5#3
Sp042	Eo00199	<i>E. spnr. azafranata</i>	676	Q5#3
Sp042	Eo00266	<i>E. spnr. azafranata</i>	635	Q5#3
Sp043	Eo00281	<i>E. spnr. azafranata</i>	676	Q3#3
Sp043	Eo00291	<i>E. spnr. azafranata</i>	676	Q3#3
Sp043	Eo00310	<i>E. spnr. azafranata</i>	676	Q2#3
Sp043	Eo00312	<i>E. spnr. azafranata</i>	676	Q2#3
Sp044	Eo00026	<i>E. escamata</i>	676	Q2#3
Sp044	Eo00035	<i>E. escamata</i>	676	Q2#2
Sp045	Eo00006	<i>E. burla</i>	676	Q3#3
Sp045	Eo00061	<i>E. burla</i>	650	Q3#3
Sp045	Eo00080	<i>E. burla</i>	676	Q2#2
Sp045	Eo00084	<i>E. burla</i>	674	Q2#3
Sp045	Eo00121	<i>E. burla</i>	676	Q5#3
Sp045	Eo00156	<i>E. burla</i>	664	Q2#3
Sp045	Eo00343	<i>E. burla</i>	676	KP1-VI
Sp046	Eo00188	<i>E. spnr. adimaria</i> 399	676	Q5#3
Sp046	Eo00287	<i>E. spnr. adimaria</i> 399	676	Q3#3
Sp047	Eo00189	<i>E. spnr. borrata</i>	676	Q5#3
Sp047	Eo00333	<i>E. spnr. borrata</i>	676	Q5#2
Sp047	Eo00388	<i>E. spnr. borrata</i>	676	Q5#1
Sp048	Eo00348	<i>E. spnr. yvatta</i> 400	676	BC-VI
Sp048	Eo00350	<i>E. spnr. yvatta</i> 400	676	BC-VI
Sp049	Eo00012	<i>E. spnr. golosata</i>	676	Q3#3
Sp049	Eo00157	<i>E. spnr. golosata</i>	676	Q2#3
Sp049	Eo00159	<i>E. spnr. golosata</i>	658	Q3#2
Sp049	Eo00240	<i>E. spnr. golosata</i>	676	Q5#2
Sp049	Eo00271	<i>E. spnr. golosata</i>	658	Q3#3

Sp049	Eo00321	<i>E. spnr. golosata</i>	676	Q3#2
Sp049	Eo00330	<i>E. spnr. golosata</i>	676	Q3#3
Sp050	Eo00028	<i>E. spnr. golosata</i>	676	Q3#2
Sp050	Eo00288	<i>E. spnr. golosata</i>	676	Q3#3
Sp050	Eo00305	<i>E. spnr. golosata</i>	676	Q3#3
Sp050	Eo00307	<i>E. spnr. golosata</i>	676	Q5#2
Sp051	Eo00010	<i>E. spnr. margarita</i>	676	Q3#3
Sp051	Eo00037	<i>E. spnr. margarita</i>	665	Q2#1
Sp051	Eo00302	<i>E. spnr. margarita</i>	676	Q5#1
Sp051	Eo00378	<i>E. spnr. margarita</i>	676	4a
Sp052	Eo00002	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00007	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00009	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00011	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00049	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00051	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00075	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00076	<i>E. spnr. margarita</i>	652	Q3#3
Sp052	Eo00151	<i>E. spnr. margarita</i>	676	Q2#3
Sp052	Eo00172	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00212	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00220	<i>E. spnr. margarita</i>	676	Q3#2
Sp052	Eo00236	<i>E. spnr. margarita</i>	658	Q5#1
Sp052	Eo00268	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00285	<i>E. spnr. margarita</i>	676	Q3#3
Sp052	Eo00355	<i>E. spnr. margarita</i>	676	4a
Sp053	Eo00021	<i>E. sp. 409</i>	676	Q3#1
Sp053	Eo00063	<i>E. sp. 409</i>	676	Q2#2
Sp053	Eo00128	<i>E. sp. 409</i>	676	Q2#3
Sp054	Eo00071	<i>E. spnr. borrata</i>	636	Q5#2
Sp054	Eo00092	<i>E. spnr. borrata</i>	676	Q2#3
Sp054	Eo00130	<i>E. spnr. borrata</i>	635	Q2#3
Sp054	Eo00282	<i>E. spnr. borrata</i>	658	Q3#3
Sp054	Eo00284	<i>E. spnr. borrata</i>	658	Q3#3
Sp054	Eo00327	<i>E. spnr. borrata</i>	635	Q2#3
unknown	Eo00342		635	BC-1

**Table A6.** Outgroup sequences for neighbor-joining analysis of barcoding sequences taken from Genbank.

Taxon	Genbank acc.		
	No.	Length	Reference
<i>Cladara limitaria</i>	AF549648	617	Hebert (2003a)
<i>Eulithis diversilineata</i>	AF549682	617	Hebert (2003a)
<i>Eulithis molliculata</i>	AF549683	617	Hebert (2003a)
<i>Euphyia unangulata</i>	AF549639	617	Hebert (2003a)
<i>Eupithecia annulata</i>	AF549656	617	Hebert (2003a)
<i>Eupithecia assimilata</i>	AF549658	617	Hebert (2003a)
<i>Eupithecia mutata</i>	AF549660	617	Hebert (2003a)
<i>Eupithecia palpata</i>	AF549655	617	Hebert (2003a)
<i>Eupithecia ravocostaliata</i>	AF549657	617	Hebert (2003a)
<i>Eupithecia subfuscata</i>	AF549659	617	Hebert (2003a)
<i>Heterophleps triguttaria</i>	AF549674	617	Hebert (2003a)
<i>Hydriomena perfracta</i>	AF549661	617	Hebert (2003a)
<i>Hydriomena transfigurata</i>	AF549638	617	Hebert (2003a)
<i>Lobophora nivigerata</i>	AF549636	617	Hebert (2003a)
<i>Orthonama centrostrigaria</i>	AF549643	617	Hebert (2003a)
<i>Orthonama obstipata</i>	AF549642	617	Hebert (2003a)
<i>Xanthorhoe ferrugata</i>	AF549641	617	Hebert (2003a)
<i>Xanthorhoe munitata</i>	AF549665	617	Hebert (2003a)
<i>Xanthorhoe packardata</i>	AF549644	617	Hebert (2003a)

## Curriculum vitae

*Name:* Patrick Strutzenberger

*Date of birth:* 07/22/1980

*Place of birth:* Vienna, Austria

### *Education*

1996-2001: Higher technical school for corporate management with special focus on quality and environmental management.

2002-04/2009: Studies of Molecular Biology at the University of Vienna with major in Zoology/Ecology.