

Molecular phylogeny, classification, and biogeographic origin of *Callerebia* and other related Sino-Himalayan genera (Insecta: Lepidoptera: Nymphalidae: Satyrinae)

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Summary

The Sino-Himalayan subregion is a widely extended transition zone between the Oriental Region and the Palaearctic Region. A most interesting feature of this area is the unique nature of many of the animal and plant taxa characteristic of its biota. In fact, the Sino-Himalayan subregion although representing the transition zone between broadly overlapping elements from two zoogeographical regions, is also the abode of several endemic plant and animal taxa. This feature is well testified by the butterfly fauna which includes several speciose genera typical of this area.

Employing DNA sequences (471 bp) of the elongation factor nuclear gene (EF-1 α) as molecular marker, the present study is aimed to investigate the phylogenetic relationships of five genera of the tribe Satyrini (Satyrinae, Nymphalidae), namely *Callerebia*, *Argestina*, *Hemadara*, *Loxerebia*, (from now on this group of genera will be named "callerebias") and *Eugrumia*, most of them consisting of extremely localized species. So far, these butterflies have never been included in molecular studies, since most of the species are rare and/or geographically restricted. Results from this study, although preliminary, draw attention to the need for a systematic revision of the whole tribe Satyrini. In particular, our results lead to reconsider the placement of all the "callerebias" genera that should be moved from subtribe Ypthimina to Erebiina.

In addition, the "callerebias" are recognized as more related to the strictly Holarctic genus *Erebia* rather than to the tropical *Ypthima*, almost certainly of Oriental origin. Thus, it is possible to hypothesize the origin of the "callerebias" from an ancient stock of Palaearctic butterflies. Ecological specialization and adaptive radiation might have driven early speciation events of the "callerebias" genera.

Zusammenfassung

Die Sino-Himalayische Unterregion ist eine breite Übergangszone zwischen der orientalischen und paläarktischen Region. Eines der interessantesten Merkmale dieses Gebietes ist die Einzigartigkeit der für seine Lebensräume typischen Tier- und Pflanzenarten. Obgleich die Region eigentlich den Übergang zwischen zwei tiergeographischen Großregionen darstellt, wird sie doch von zahlreichen endemischen Tier- und Pflanzentaxa besiedelt. Diese Eigenschaft wird gut durch die Schmetterlingsfauna unterstützt, welche in der Region einige typische Gattungen aufweist.

Unter Nutzung von nuklearen DNA-Sequenzen mit einer Länge von 471 Basenpaaren (Elongation factor EF-1 α) als molekulare Marker untersucht die vorliegende Arbeit die phylogenetischen Beziehungen von fünf Gattungen des Tribus Satyrini (Satyrinae, Nymphalidae): *Callerebia*, *Argestina*, *Hemadara*, *Loxerebia* (im folgenden "Callerebias" genannt) und *Eugrumia*. Die meisten dieser Gattungen bestehen aus lokal extrem verstreuten vorkommenden Arten.

Bislang wurden diese Schmetterlinge noch nie in molekularen Studien berücksichtigt, weil die meisten Arten selten sind und geographisch sehr beschränkt auftreten. Die noch vorläufigen Ergebnisse der Studie weisen auf die Notwendigkeit einer kompletten systematischen Revision des Tribus Satyrini hin. Insbesondere sollten die Gattungen "Callerebias" vom Subtribus Ypthimina zum Subtribus Erebiina verschoben werden.

Weiterhin ist erkennbar, daß die "Callerebias" näher mit der strikt holarctischen Gattung *Erebia* verwandt sind als mit der Gattung *Ypthima*, welche höchstwahrscheinlich orientalischen Ursprungs ist. Deshalb kann die Hypothese aufgestellt werden, daß die "Callerebias" aus einer ursprünglichen Gruppe paläarktischer Schmetterlinge abstammen. Die frühe Artbildung innerhalb der Gruppe wurde vermutlich durch eine Radiation und ökologische Spezialisierung beschleunigt.

Key words: Himalayan butterflies, molecular phylogeny, classification, Lepidoptera, *Callerebia*, *Argestina*, *Hemadara*, *Loxerebia*, *Eugrumia*

Introduction

The Sino-Himalayan subregion is a widely extended transition zone between the Palaearctic and Oriental region. The borders between these two regions are indeed very faded. WALLACE (1876), in his study on "Geographical distribution of Animals", observed "It is perhaps less clear whether the Palaearctic (region) should be separated from the Oriental region, with which it has undoubtedly much in common, but there are many and powerful reasons for keeping it distinct." A most interesting feature of this area is the unique nature of many of the animal and plant taxa characteristic of its biota. In fact, the Sino-Himalayan subregion whilst represents the transition zone between broadly overlapping elements from two zoogeographical regions, is also the abode of several endemic plant and animal taxa (JOHANSSON et.al. 2007, MARTENS et.al. 2006). Both palaeogeographic events, leading to the formation of the Himalayan chain and adjacent territories, and a composite variety of climatic and environmental conditions may explain the present biodiversity feature of this district. The continuous rise of the Himalayan range and Tibetan plateau generated eastwards a landscape marked by ridges of high mountains with a north-south trend, interrupted eastwards by deep gorges generated by the rivers Brahmaputra, Irrawadi, Salween, Mekong and Yangtze. In addition, the formation of a climate of monsoonal type has

deeply influenced the configuration of both animal and plant populations. The palaeogeographic and climatic historical events produced natural refuges for the preservation of many plants that today are relics of the Tertiary (ZHU & YAN 2002), and the creation of new habitats opened the possibility for adaptive radiation of new species. Typical events of this spectacular process of diversification are well known in plants, for instance in the genera *Genziana*, *Rhododendron*, *Primula*, *Androsace* and *Saussura* (ZHANG et.al. 2007). The uniqueness of Sino-Himalayan subregion is also testified by the butterfly fauna which includes several genera typical of this area, often represented by several species, most of them being confined to restricted ranges, particularly in the Hengduan range, and in other mountain areas of South-West China such as *Byasa*, *Bhutanitis*, *Teinopalpus*, *Aporia*, *Chonala*, *Neope*, *Rhaphicera*, and many others.

In this study, we focused our interest on five genera of Satyrinae (Nymphalidae), endemic of the Sino-Himalayan subregion and mainly consisting of extremely localized species, namely *Callerebia*, *Argestina*, *Hemadara*, *Loxerebia*, (from now on this group of genera will be named "callerebias") and *Eugrumia*. All these butterflies fly from low altitudes, in tropical and montane areas, to high altitudes in alpine habitats. Within "callerebias", genus *Callerebia* (nine species) ranges

from Kashmir to South West China, with a single taxon (*C. polyphemus*) reaching Central China (Fig.1); ecological requirements of *Callerebia* species are quite variable, they occur from temperate valley and lowlands at an altitude of 1000 m up to 3000 m. Instead, the genus *Argestina* includes four high altitude adapted species restricted to Tibet, usually found between 4000 and 5000 m. Species of the genera *Loxerebia* (thirteen species) and *Hemadara* (seven species), formerly unified in the genus *Loxerebia*, inhabit Sino-Himalayan eastern areas, where *Loxerebia* species extend northwards and *Hemadara* ones southwards (Fig.1); they fly at altitudes between 2000 and 3500 m. Finally, the three species belonging to the newly described genus *Eugrumia* (DELLA BRUNA et al. 2000b), formerly assigned to genus *Paralasa*, are similar in morphology and ecology and their populations are very localized, the distribution range of this genus being limited to E Tibet, Qinghai, Sichuan, Gansu and NW Yunnan.

Satyrinae is the second largest Nymphalidae subfamily, including approximately 2400 species (ACKERY et al. 1999) distributed worldwide, except Antarctica, and adapted to a wide array of habitats, from the arctic tundra to the tropical rain forest. In spite of their species richness, Satyrinae (commonly called Satyrids) are poorly studied, and the phylogenetic relationships among genera and species, as well as their taxonomic classification, are still controversial. From a phenetic point of view, MILLER (1968) suggested a

comprehensive systematic rearrangement and classification of satyrids, based on 23 morphological characters. Later, HARVEY (1991), and ACKERY (1999) proposed further classifications just remodelling to some extent the Miller's scheme. Concerning "callerebias" and their allied taxa, DELLA BRUNA et al. (2000a, 2002) proposed a taxonomic revision based on morphological features of wing patterns and genital structures. They considered "callerebias" as belonging to subtribe Ypthimina (tribe Satyrini), as well as previously MILLER (1968) and subsequently BROWER (2006) still do. However, preliminary results of molecular phylogenetic reconstruction (SBORDONI et al., unpublished data), indicated as doubtful the systematic position of "callerebias" and suggested the need to remove the group from subtribe Ypthimina (DELLA BRUNA et al. 2002).

Recently, a number of researchers carried out molecular studies addressed to reconstruct phylogenetic relationships of Satyrinae. The latest and most comprehensive molecular investigation was performed by PEÑA et al. (2006); they analysed 165 Satyrinae taxa by means of DNA sequences of nuclear (Elongation Factor-1 α and *wingless*) and mitochondrial (cytochrome oxidase I) genes. Nevertheless, right now, no molecular study has been addressed to the "callerebias", probably because they are rare, often geographically localized, and with most museum samples old and inappropriate for DNA studies.

Employing DNA sequences of the elongation factor nuclear gene (EF-1 α) as molecular marker, the aim of the present study to investigate the phylogenetic relationships of the five

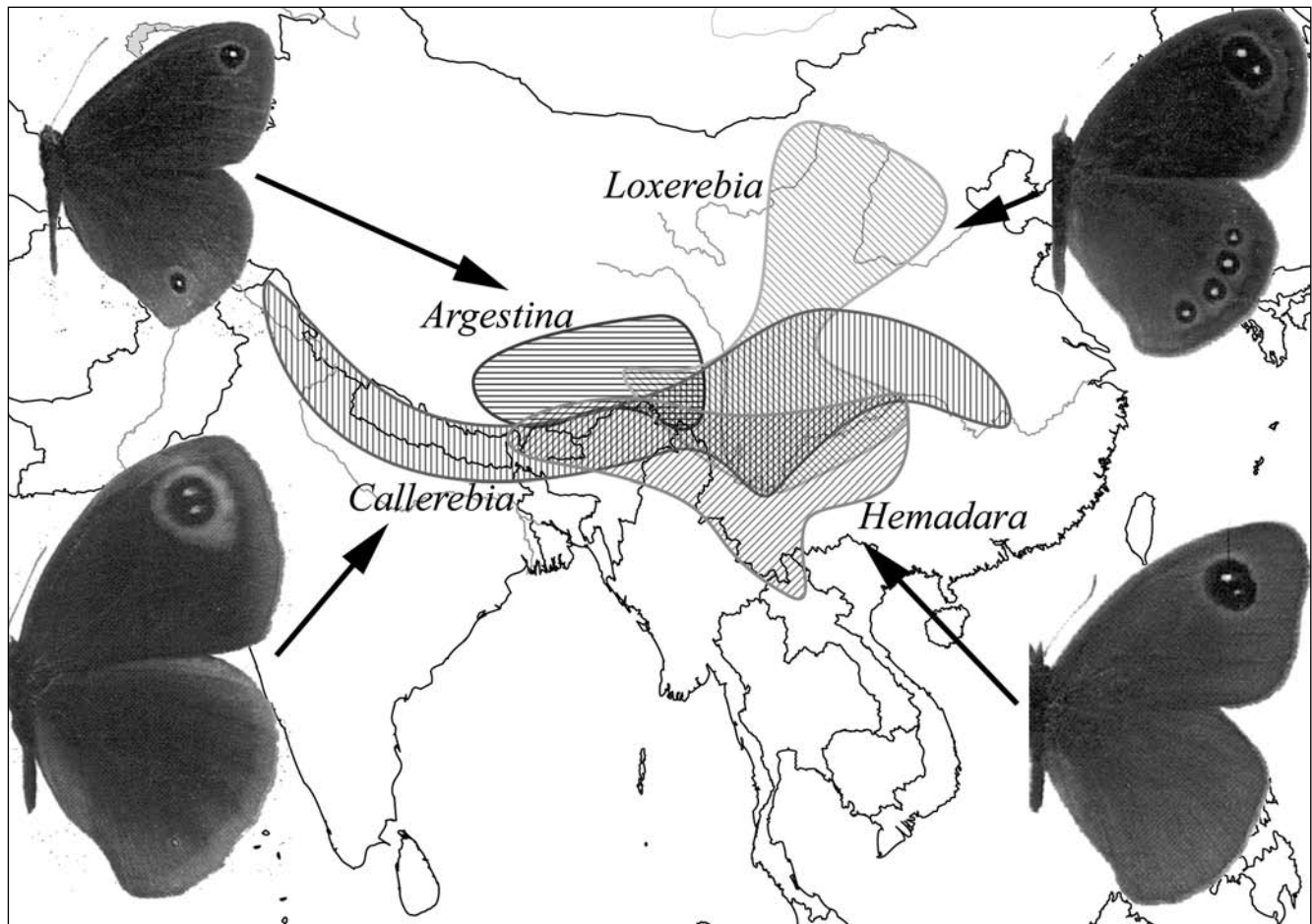


Figure 1: Distribution ranges of the Sino-Himalayan "callerebias", i.e. satyrid genera *Callerebia*, *Argestina*, *Hemadara* and *Loxerebia*.

mentioned Satyrinae genera. This preliminary report deals with a limited number of representative species, and attempts to identify their most likely geographic origin (Palearctic vs. Oriental), by analysing additional hypothetical sister taxa, such as species belonging to genera *Erebia*, *Paralasa* and *Ypthima*. Actually, *Erebia* and *Paralasa* are typically Holarctic genera, while *Ypthima* is a speciose genus, largely tropical, widespread in the Oriental, Afrotropical and Australian Regions.

Materials and methods

We analysed DNA sequences of the EF-1 α nuclear gene from twenty-one specimens belonging to nineteen species, representing thirteen genera of the subfamily Satyrinae. Additionally, one DNA sequence from *Heliconius hecale* (Nymphalid subfamily Heliconinae), has been analysed and

utilized as outgroup. In Table 1 are reported the collecting locality for each specimen and its current systematic position according to BROWER (2006). Study samples are currently preserved in the Sbordonì's collection at Tor Vergata University.

DNA was extracted from one or two legs of dried specimens by mechanical grinding of the tissue, followed by chemical digestion with proteinase K and two chloroform extractions and ethanol precipitations. The resulting DNA was re-suspended in sterile water and maintained frozen at -70°C until further use. For each specimen we sequenced 471 bp of the EF-1 α gene. DNA amplification was performed by using primers available in CHO et al., (1995), i.e. M3 (5' CACATYACATTGTCGTSATYGG 3') and rcM51 (5'CATGTTGTCGCCGTGCCAAC 3'). Standard DNA amplification reaction was set using 0.5-1 μ l of DNA template

Table 1: List of the specimens used for molecular studies, their systematic position, according to BROWER (2006), and collecting localities.

Classification	collecting locality
subfamily Satyrinae	
tribe Satyrini	
subtribe Ypthimina	
<i>Argentina inconstans</i> (South, 1913)	China, Tibet. Road Lasha Nymchi, 20 Km E of Mi La, 4150m, 04.VII.2000
<i>Argentina pomena</i> (Evans, 1915)	China, Tibet. Nyang Chu River, 14-20 Km east Bayi, 3150m, 10.VII.2000
<i>Argentina waltoni</i> (Elwes, 1906)	China, Tibet. Drowolung Valley, vic Nakar, 4000m, 21-29.V.2002
<i>Boeberia parmenio</i> (Böber, 1809)	China, Qingai. 15 Km S Huang River, 2600-3400m, 09.VII.1997
<i>Callerebia nirmala</i> (Moore, 1865)	India, Himachal Pradesh. Manikaran, 2200m, 12.VII.2001
<i>Callerebia scanda</i> (Kollar, 1886)	India, Himachal Pradesh. Manali, 2500m, 09.VIII.2001.
<i>Hemadara narasingha</i> (Moore, 1857)	India, Laos. Xam Nua. III.2003
<i>Loxerebia bocki</i> (Oberthür, 1893)	China, Sichuan. 40km N Wenchuan 1800m, 31°46'20"N-103°42'51"E, 01.VIII.2006
<i>Loxerebia saxicola</i> (Oberthür, 1876)	China, Shanxi. Tschingling Mts., Taibeichan Nat. res., 2000m, 05-15.VIII.2005
<i>Paralasa jordana</i> (Staudinger, 1882)	Tajikistan. Alai Mts., Jordan village, m 2000, 18.VI.1995.
<i>Ypthima confusa</i> (Shirôzu & Shima, 1977)	Vietnam, North. Hoang-Lien-Son-Prou. Sa-Pa, 12-17.V.1990.
<i>Ypthima huebneri</i> (Kirby, 1871)	India, Chattisgarh. Kranger Valley. Jagol Alpur, 01.XII.2004
subtribe Erebiina	
<i>Erebia aethiops uralensis</i> (Goltz, 1930)	Russia, S-Ural. Orenburg reg., 14-15.VII.2004.
<i>Erebia cyclopius aporia</i> (Schawerda, 1919)	Russia, Amur. Kuldu, 600m, 9-14.VI.2002
<i>Proterebea afra afra</i> (Fabricius, 1787)	Russia, SW Siberia. Orenburg reg., Kuvandyk env., 14.V.2003
<i>Proterebea afra krimea</i> (Sheljuzhko, 1929)	Ukraine, Crimea. Sudak env., 03.V.2003
uncertain position	
<i>Eugrumia koenigi bozanoi</i> (Della Bruna, Gallo, Lucarelli & Sbordonì, 2000)	China, Tibet. Nyingtri prov., May-La, 4300m. 30.VI.1997
<i>Eugrumia koenigi bozanoi</i> (Della Bruna, Gallo, Lucarelli & Sbordonì, 2000)	China, E-Tibet. Qamdo Valley, 3200-3500m, VI.2000
tribe Elymniini	
<i>Elymnius hypermnestra</i> (Linnaeus, 1763)	India, Chattisgarh. Jagdalpur, XII.2004
tribe Melanitini	
<i>Melanitis leda</i> (Linnaeus, 1758)	India, Chattisgarh. Raipur, 29.XII.2004
tribe Zetherini	
<i>Penthema darlisa</i> (Moore, 1879)	China, W-Yunnan. Salween Valley, 1300m, 09.VI.2004
subfamily Heliconinae	
<i>Heliconius hecale</i> (Fabricius, 1775)	Colombia. Cordillera de las Quinchas, P.to Boyaca-Otanche 300-1000m.

with 12,5 µl of PCR-Master Mix (Promega) and 0,25 µl of each primer solution (0,5 µg/µl) in a total volume of 25 µl and the polymerase chain reaction (PCR) was carried out in a Perkin Elmer™ GeneAmp PCR System 9700. Samples were initially denatured at 95 °C for 2 min followed by 35 cycles of amplification (denaturation at 95 °C for 30 s, annealing at 47°C for 15 s for five cycles and 52° for 30 s for thirty cycles, extension at 72 °C for 2 min) with a final extension at 72 °C for 10 min. When a nested subsequent amplification reaction of the PCR product was needed, we used two internal primers from CHO et al. (1995), i.e. M44 (5' GCTGAGCGYGARCGTGGTATCAC 3') and rcM44 (5' CTTGATGAAATCYCTGTGTCC 3') with the same procedure used for early PCR, except an extension time of 35s for cycle. Afterwards PCR products were purified applying ExoSAP-IT enzymatic treatment (USB Corporation). Cleaned DNA products were cycle-sequenced using fluorescent dye-labelled terminators (ABI Big Dye Terminator Cycle Sequencing Kit, Applied Biosystems, Perkin-Elmer). In order to minimize possible ambiguities, we sequenced both DNA strands in an ABI Prism™ 3100 sequencer and aligned the fragment with Sequencer 4.1 software (1999-2000, Genes Code Corporation, Ann Arbor, Michigan). A manual alignment of forward and reverse strands made possible to control the exact complementarity of the filaments and correct any imperfections and ambiguities due to deficiency in reaction sequence processes.

A Bayesian approach was used to analyse the DNA sequence dataset. We used the software "MrBayes" (RONQUIST & HUELSENBECK 2003), which is a program for the Bayesian estimation of phylogeny. Bayesian inference of phylogeny is based upon a quantity called the posterior probability distribution of trees, which is the probability of a tree conditioned on the observations. MrBayes uses a simulation technique called Markov chain Monte Carlo (MCMC) to approximate the posterior probability of trees.

The appropriate model of DNA substitution was determined using the software MODELTEST version 3.06 (POSADA & CRANDALL 1998). This method employs a hierarchical likelihood ratio test to determine the model that best fits the data, and the parameters for the chosen model (base composition, substitution rates, proportion of invariable sites and gamma shape parameter). Bayesian analysis was performed using four search chains for 1000000 generations, sampling trees every 100 generation. The first 800 samples were discarded as burn-in based on a visual inspection of when log likelihood values reached stationary. Parameter stability was estimated by plotting log-likelihood values against generation time, and a consensus tree with posterior probabilities was then generated in PAUP* 4.0b10 (SWOFFORD 1998).

Results

The obtained data set of nucleotide sequences consists of 471 bp from EF1- α nuclear gene for each of the 23 specimens. On the basis of this data set, the program MODELTEST suggested the model of TAMURA & NEI (1993) with among-site rate heterogeneity and proportion of invariant sites (TrN+I+G) as the most suitable for our data.

The consensus tree resulting from the Bayesian analysis is

outlined in Figure 2. A main, distal, most likely monophyletic group of taxa may be observed; it includes the "callerebias" group (genera *Argentina*, *Callerebia*, *Hemadara* and *Loxerebia*), with the addition of *Proterebia afra* as the sister taxon of *Argentina* species. Within this large and statistically well supported (posterior probability value > 0.99) cluster, each of the genera of "callerebias" forms a monophyletic subcluster of species well supported by significant values of posterior probability (> 0.95), except for the genus *Callerebia* (posterior probability value = 0.84). Just outside the "callerebias" cluster, *Paralasa jordana* sets out with a node value of 0.91. Species not included in tribe Satyrini (*Melanitis leda*, *Elymnias hypermnestra*, *Penthema darlisa*, *Heliconius hecale*) apart, the tree topology reveals another main cluster related to "callerebias" + *Paralasa* group. This cluster is not statistically significant (posterior probability value = 0.53) and consists of two well supported subclusters (posterior probability value > 0.95), a first one including all *Erebia* species with the addition of *Boeberia parmenio*, and a second one including the two *Eugrumia koenigi* sequences. Ultimately, the Bayesian analysis on EF1- α gene sequences put together all the species groups so far mentioned in a single clade with a statistically significant support (P=0.96), but keeps out the *Ypthima* clade (two species; posterior probability value > 0.94). The tree analysis recognizes a unequivocal global cluster of the species included in the tribe Satyrini (posterior probability value > 0.99), but does not allow to solve, with a statistically significant support, positions concerning the taxa *Melanitis leda*, *Elymnias hypermnestra* and *Penthema darlisa*, belonging to the tribes Melanitini, Elymniini, and Zetherini, respectively.

Discussion

Transition zones are belts of biogeographic ambiguity where biotas evolved independently, overlapping and merging to various extents along relatively wide areas. The Sino-Himalayan transition zone is of special interest because, besides the obvious overlap between Palaearctic and Oriental biotas, is characterised by several taxa peculiar to this region. Among butterflies, the genera *Callerebia*, *Loxerebia*, *Argentina*, *Hemadara* and the recently erected genus *Eugrumia*, meet these requirements. The "callerebias" genera seem to represent a monophyletic group including butterflies ranging in altitude from lowlands (*Callerebia*) to 5000 metres in the Tibetan plateau (*Argentina*). *Argentina* species show the size, colour and wing pattern typical of Palaearctic *Erebia*, while, in the remaining genera, an ocellate pattern *Ypthima*-like is predominant. Most current classifications include these genera within the Ypthimini tribe, encompassing the wide majority of taxa typical of the Oriental Region. At present, one of the most reliable website concerning the systematic arrangement of life organisms is "Tree of Life web project", and browsing the Satyrinae web-pages, authored by N. Wahlberg and A.V.Z. Brower, all the "callerebias" genera are still listed as belonging to the subtribe Ypthimina (BROWER, 2006). As already outlined, the only exhaustive study concerning these genera is a taxonomic revision based on morphological features of wing patterns and genital structures. In the first edition of the study (DELLA BRUNA et al. 2000a), the authors fol-

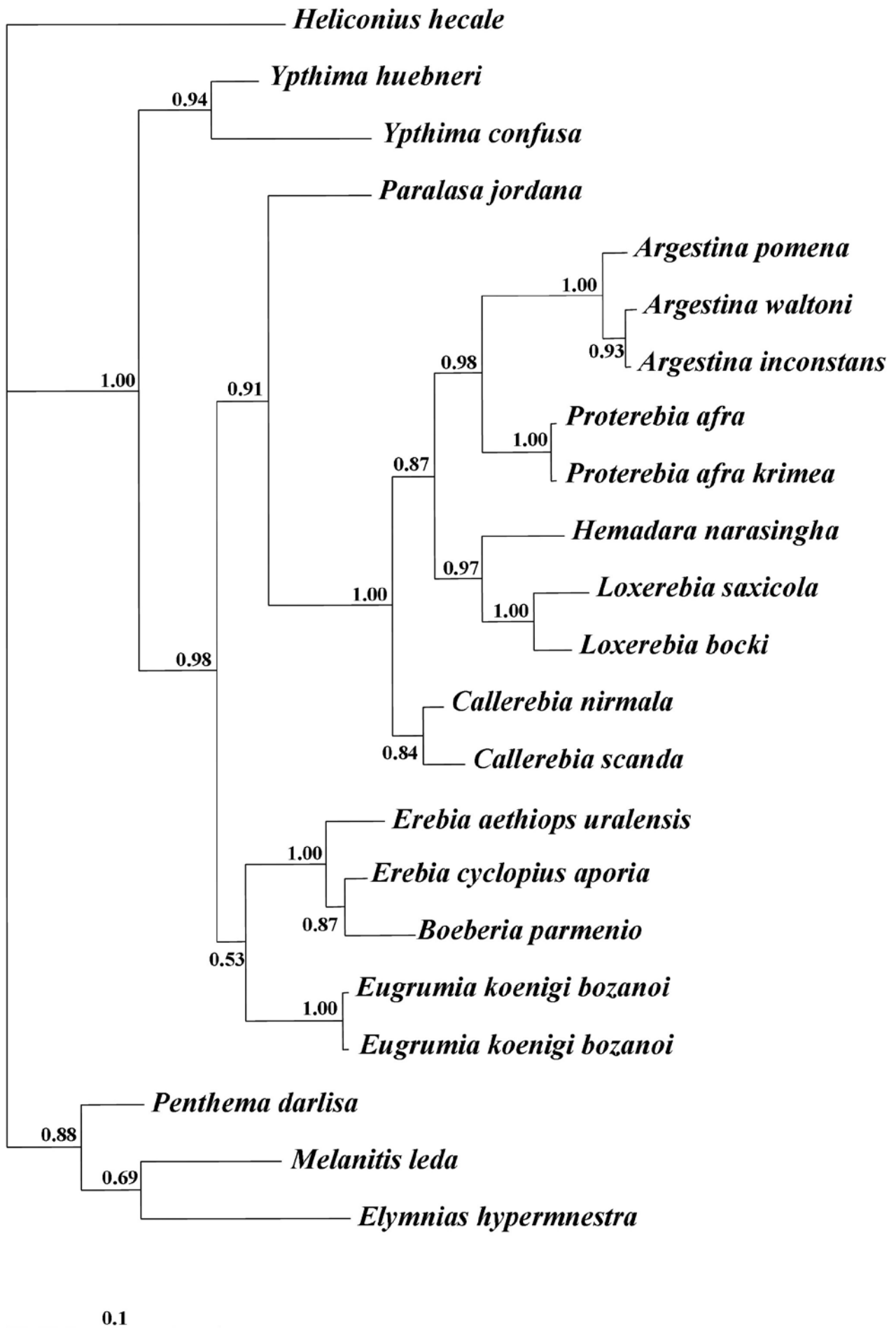


Figure 2: Molecular phylogenetic hypothesis based on Bayesian analysis. Numbers given above branches are posterior probabilities.

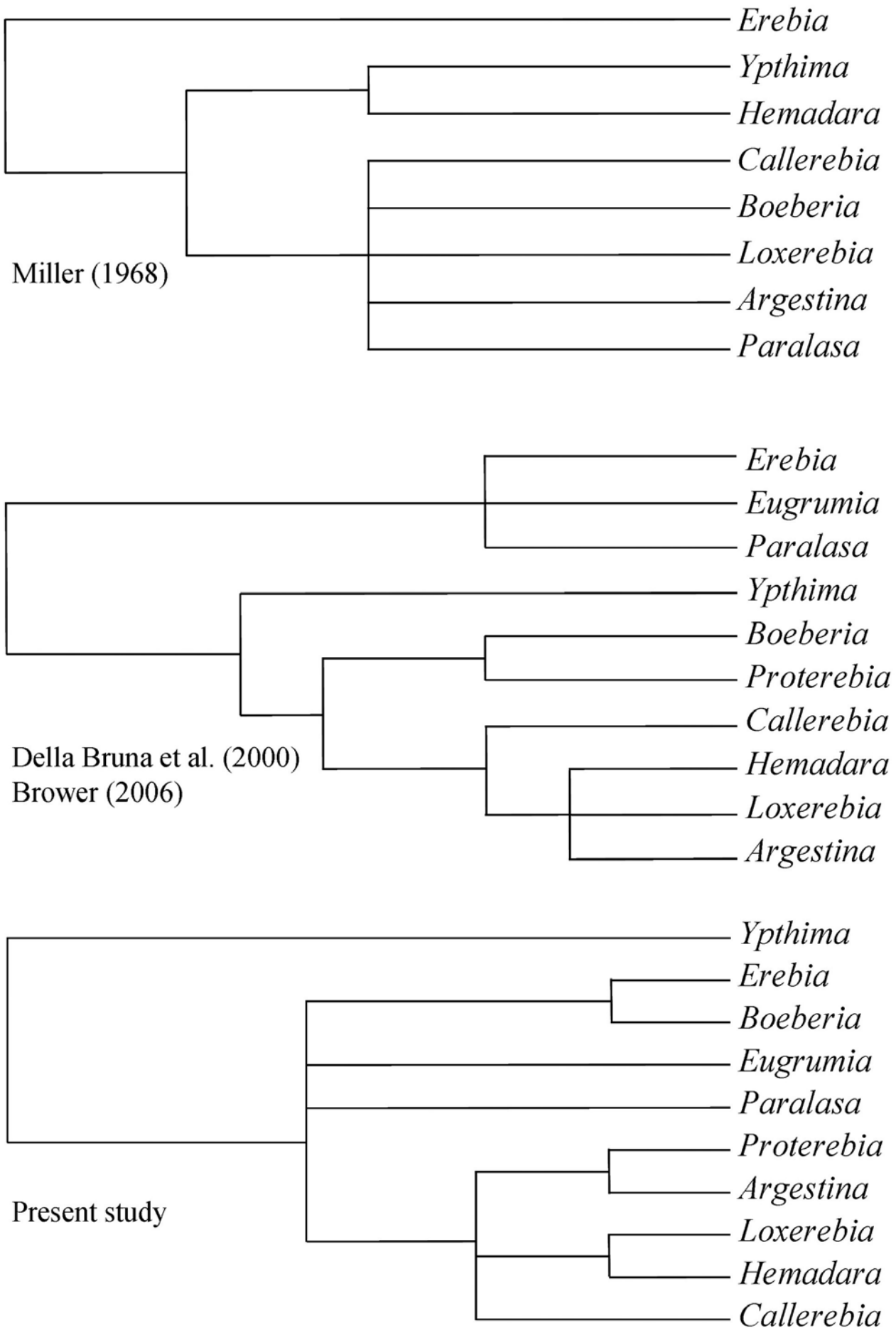


Figure 3: Comparison among three different systematic hypotheses for "callerebias" and allied genera.

lowed the mainstream hypothesis, placing the “callerebias” in the Miller’s subtribe Ypthimina. However, in the second revised edition, they suggested, based on our preliminary results from molecular data, to remove the “callerebias” genera from subtribe Ypthimina.

So far, these butterflies have never been included in molecular studies on Satyrinae, since most of the species are rare and/or geographically restricted. In the present study, analyses of DNA sequences from the EF-1 α nuclear gene in twenty-two samples generated a gene tree (Fig. 2) having, in most cases, a statistically significant support both to distal and basal nodes of the molecular phylogeny. Even if the position of outgroup species sequences, *Elymnias hypermnestra*, *PentHEMA darlisa*, and *Melanitis leda*, remains unresolved, congeneric species form statistically well supported clusters, with the exception of *Callerebia* (2 species assayed).

The overall genera *Callerebia*, *Argestina*, *Proterebia*, *Hemadara* and *Loxerebia* compose a substantiated monophyletic clade, validating their close relationships. In particular, *Hemadara narasingha* emerges strictly related to the two assayed *Loxerebia* species. In addition, *Proterebia afra*, already included in the subtribe Erebiina (BROWER 2006), appears to be clearly related to the “callerebias”. In particular, *Proterebia afra* stands as the sister taxon of *Argestina*. Actually, WARREN (1936), based on male genitalia morphology, hypothesised that *Proterebia afra* (earlier *Erebia phegea*) is to some extent more related to *Callerebia* than to the genus *Erebia*. Correspondingly, *Boeberia parmenio*, a monotypic genus formerly included in the subtribe Ypthimina, appears integrated in a well-supported clade together with the two assayed *Erebia* species (subtribe Erebiina), linking to the Asian species *E. cyclopius*.

Looking at more basal nodes of the gene tree, *Paralasa jordana* joins directly to the monophyletic “callerebias” clade, although without significant statistical support, hence questioning the placement of *Paralasa* in the subtribe Ypthimina (BROWER 2006).

Despite an apparent link (not statistically significant) to the *Erebia-Boeberia* clade, the cluster consisting of two *Eugrumia* species, formerly described under the genus *Erebia* and further assigned to *Paralasa* (D’ABRERA 1990), represents the fourth branch of a large clade involving the “callerebias”, *Paralasa*, *Eugrumia* and *Erebia* groups.

Consistently enough, the *Ypthima* species group appears placed outside this large clade with a highly significant value of posterior probability (0.96).

The main outcome of this molecular study is that the systematic position of the “callerebias” and their allied species disagrees with the former classifications (MILLER 1968, DELLA BRUNA et al. 2000a, BROWER 2006), as summarized in Figure 3. The genera *Callerebia*, *Argestina*, *Hemadara*, *Loxerebia*, *Paralasa* and *Eugrumia* were so far included, with *Ypthima*, in the subtribe Ypthimina, whereas the molecular phylogenetic reconstruction clearly suggests that the whole group is more allied to *Erebia* (subtribe Erebiina).

from Ypthimina to subtribe Erebiina, together the genetically related *Proterebia afra*. Likewise, the genera *Boeberia* and *Paralasa*, formerly placed in subtribe Ypthimina, should now be placed in the subtribe Erebiina.

Another aim of this paper was to solve the issue of the geographic origin of the “callerebias”, as a typical Sino-Himalayan taxon. The phylogenetic trees obtained in this study offer some advice in this regard. We showed that they are more related to the strictly Holarctic genus *Erebia* rather than to the tropical *Ypthima*, almost certainly of Oriental origin. Thus, it is possible to hypothesize their ancient origin from an ancient stock of Palaearctic butterflies. Following the critical geo-climatic changes of Sino-Himalayan region, they would then have locally experienced repeated episodes of speciation, possibly by filling empty niches locally available. They could have radiated into a wide array of environments, from high elevation, steppic habitats, preferred by the black, small-sized *Argestina* species, down through lower submontane, tropical habitats, where the largest species of *Callerebia* occur.

However, further studies are needed to: i) clarify the species-level taxonomic relationships within the “callerebias” and allied taxa and ii) better define the phylogeny within the tribe Satyrini. Researches currently carried out in our lab, based on a much wider data set, address to uncover the evolutionary scenario where the “callerebias” originated, including attempts to date the major bulks of cladogenetic events in the Sino-Himalayan area.

Acknowledgments

This research has been supported with grants from the Tor Vergata University to the senior author (V.S.). We acknowledge Caterina Fani for her early involvement in the molecular phylogeny studies in our lab, and Marco Lucarelli for taxonomic advice.

Results from this study, although preliminary, draw attention to the need for a systematic revision of the whole tribe Satyrini. In particular, these outcomes lead to reconsider the placement of all the “callerebias” genera that should be moved

References

- ACKERY, P.R.; R. DE JONG & R.I. VANE-WRIGHT (1999): The butterflies: Hedyloidea, Hesperioidea, and Papilionoidea. Lepidoptera: Moths and Butterflies. 1. Evolution, Systematics, and Biogeography. - Handbook of Zoology 4: 263-300.
- BROWER, A.V.Z. (2006): Ypthimina Reuter 1896. Version 28 November 2006 (temporary). - <http://tolweb.org/Ypthimina/70806/2006.11.28> in The Tree of Life Web Project, <http://tolweb.org/>.
- CHENG-YIH, W. (1998): Delineation and Unique Features of the Sino-Japanese Floristic Region. - Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China.
- CHO, S.; A. MITCHELL, J. REGIER, C. MITTER, R.W. POOL, T.P. FRIEDLANDER & S. ZHAO (1995): A highly conserved nuclear gene for low-level phylogenetics: elongation factor-1 α recovers morphology-based tree for heliothine moths. - Molecular Biology and Evolution 12: 650-656.
- D'ABRERA, B. (1990): Butterflies of the Holarctic Region. Part 1: Papilionidae, Pieridae, Danaidae & Satiridae. - Victoria: Hills House.
- DELLA BRUNA, C.; E. GALLO, M. LUCARELLI & V. SBORDONI (2000a): Guide to the butterfly of the Palearctic region. Satyrinae part II, - First Edition. Ed. G.c.Bozano, Omnes Artes, Milano.
- (2000b): *Eugrumia*, a new name for *Grumia* (Lepidoptera: Satyridae, Satyrinae, Ypthimini). - Fragmenta entomologica 32: 297-298.
- (2002): Guide to the butterfly of the Palearctic region. Satyrinae part II. - Second Edition - Ed. c.Bozano, Omnes Artes, Milano .
- ELLENBERG, H. (1982): Die Vegetation Mitteleuropas mit den Alpen. - Stuttgart, Ulmer, 3. Aufl., 989 S.
- HARVEY, D. J. (1991): Higher classification of the Nymphalidae, Appendix B. - In: NIJHOUT, H.F. (ed): The Development and Evolution of Butterfly Wing Patterns. - Smithsonian Institution Press, pp. 255-273.
- JOHANSSON, U.S.; P. ALSTROM, U. OLSSON, P.G.P. ERICSON, P. SUNDBERG & T.D. TREVOR (2007): Build-up of the Himalayan avifauna through immigration: a biogeographical analysis of the *Phylloscopus* and *Seicercus* warblers. - Evolution 61 (2): 272-279(8).
- MARTENS J. & Y.H. SUN (2006): Evolutionary history of selected bird taxa from the Sino-Himalayan region. - Acta Zoologica Sinica 52 (Supplement) 292.
- MILLER, L.D. (1968): The higher classification, phylogeny and zoogeography of the Satyridae (Lepidoptera). - Memoirs of the American Entomological Society 24: 1-174.
- OLSSON, U.; P. ALSTRÖMB, M. GELANG, C.D. PER, G.P. ERICSON & P. SUNDBERG (2006): Phylogeography of Indonesian and Sino-Himalayan region bush warblers (*Cettia*, Aves). -Molecular Phylogenetic and Evolution 41: 556-565.
- PEÑA, C.; N. WAHLBERG, E. WEINGARTNER, U. KODANDARAMAIAH, S. NYLIN, A.V.L. FREITAS & A.V.Z. BROWER (2006): Higher level phylogeny of Satyrinae butterflies (Lepidoptera: Nymphalidae) based on DNA sequence data. - Molecular Phylogenetics and Evolution 40 (1): 29-49.
- POSADA, D. & K.A. CRANDALL (1998): Modeltest: testing the model of DNA substitution. - Bioinformatics 14: 271-275.
- RONQUIST, F. & J. P. HUELSENBECK (2003): MRBAYES 3: Bayesian phylogenetic inference under mixed models. - Bioinformatics 19: 1572-1574.
- SWOFFORD, D.L. (1998): PAUP: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0d64 ed. - Sinauer Associates, Inc. Publishers, Sunderland, MA.
- TAMURA, K. & M. NEI (1993): Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. - Molecular Biology and Evolution 10: 512-526.
- WALLACE, A.R. (1876): Geographical distribution of Animals. - McMillen & Co.
- WARREN, B.C.S. (1936): Monograph of the genus *Erebia*. - London.
- ZHANG, X.L. & Y.M. YUAN (2007): Genetic structure and differentiation of *Gentiana atuntsiensis* W. W. Smith and *G. striolata* T. N. Ho (Gentianaceae) as revealed by ISSR marker. - Botanical Journal of the Linnean Society 154: 225-232.
- ZHU, H. & L. YAN (2002): A discussion on biogeographical lines of the tropical-subtropical Yunnan. - Chinese geographical science 12, 1: 90-96.

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