The African copper connection

Apart from their colourful appearance, Copper butterflies are intriguing because of their highly disjunct distribution. Almost all species occur in the Holarctic region, but a few are found isolated in the Southern Hemisphere. To learn more about the interrelationship of the southern taxa and their distribution history, an analysis of the phylogeny of the group was carried out. Although morphological characters are sufficient to diagnose the species, they proved insufficient to build a phylogeny on. Therefore, we had recourse to molecules, using two genes. Here we report on the results with regard to the African representatives.

Rienk de Jong & Karen van Dorp

National Museum of Natural History
Naturalis
PO Box 9517
2300 RA Leiden
The Netherlands
jong@naturalis.nnm.nl

Search for the phylogeny of copper butterflies

The small copper (*Lycaena phlaeas* Linnaeus, 1758) is one of the most widely spread butterfly species in the world (figure 1). This is undoubtedly due to its high ecological adaptability. The species is found from the shores of the Arctic Ocean to Malawi in southern Africa, and from sea level to 4500 m a.s.l. It easily adapts its life cycle to local conditions. In the high north there are one or two generations per year depending on summer conditions, in the Mediterranean area there are up to four or more generations per year and the species may be on the wing throughout the year. The species belongs to the tribe Lycaenini (Lycaenidae: Lycaeninae; Bozano & Weidenhoffer 2001), a group of *c*. 80 species mainly distributed in the Holarctic Region (and largely within the range of *L. phlaeas*) and subdivided into a variable number of genera. In addition, the tribe is represented in Africa south of the Sahara by *L. phlaeas* (south to Malawi) and two species in South Africa, *L. orus* Stoll, 1780, and *L. clarki* Dickson, 1971, and surprisingly in New Zealand by four species. The other tribe of the subfamily, Heliophorini (Bozano & Weidenhoffer 2001), also has a remarkable distribution, with one genus in the Oriental region, one in Papua New Guinea and one in Guatemala. To understand these distributions we need to know the phylogeny of the group. In this paper we focus on the question whether the South African taxa are closely related to the other taxon in Subsaharan Africa, *L. phlaeas*, or if not, what their position is within the Lycaenini. As it turned out, we could not understand it without taking the entire subfamily into consideration.

Morphological characters

The use of morphological characters has led so far to a bewildering subdivision of the subfamily, and particularly the tribe Lycaenini, into genera obscuring relationships. Even the subdivision of Lycaeninae into two tribes is only weakly supported by morphological characters. Bozano & Weidenhoffer (2001) divide the Lycaenini into four Palaearctic and six non-Palaearctic genera. The Palaearctic *Lycaena* species are subdivided into six species groups, that partly correspond with genera recognized by other authors. The distinction is mainly based on the
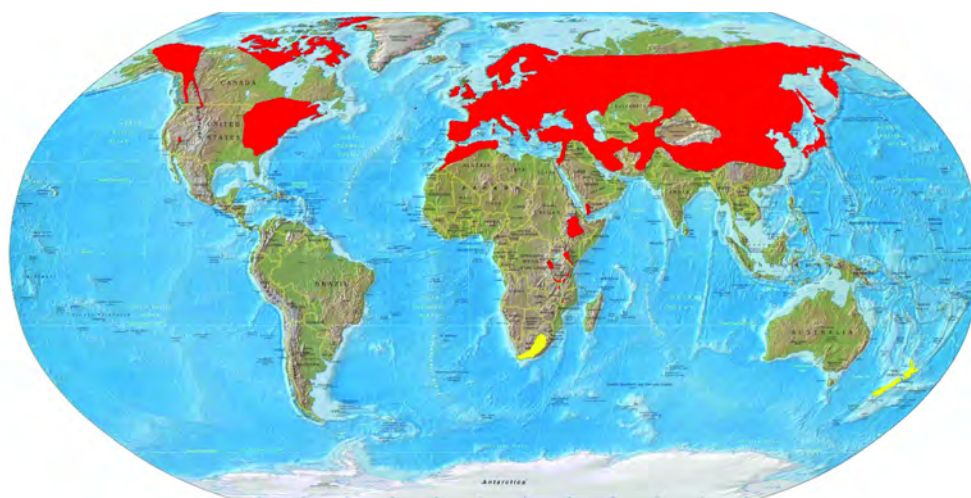

Figure 1. World distribution of the small copper (*Lycaena phlaeas*, red) and the distribution of *Lycaena* in the Southern Hemisphere (yellow). Map by courtesy of the University of Texas Libraries, The University of Texas at Austin (www.lib.utexas.edu).
*Verspreiding van de kleine vuurvlinder (*Lycaena phlaeas*, rood) en de verspreiding van* Lycaena *op het zuidelijk halfrond (geel). Kaart met dank aan de University of Texas Libraries, The University of Texas at Austin (www.lib.utexas.edu).*
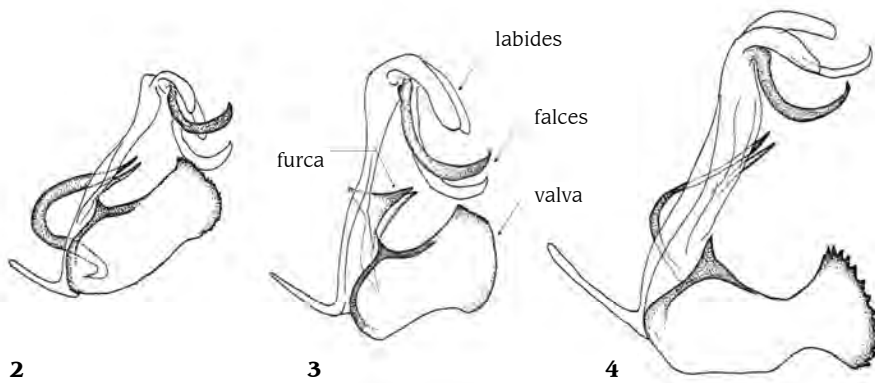
Figures 2-4. Male genitalia viewed from the left; the right valva and the aedeagus have been omitted for simplicity reason. **2** *Lycaena orus* (South Africa, Western Cape Province, nr Durbanville, 27 December 1984, H. Geertsema), **3** *L. phlaeas phlaeas* (The Netherlands, Duurwoude, 1 September 1964), **4** *L. dispar batavus* (The Netherlands, Lemsterland, September 1969, F.A. Bink). The specimens are in the collection of the National Museum of Naturalis History Naturalis, Leiden. Illustration: R. de Jong

*Mannelijke genitaliën van links gezien; de rechter valve en de aedeagus zijn weggelaten om het beeld te vereenvoudigen. **2** Lycaena orus (Zuid-Afrika, Western Cape Province, nr Durbanville, 27 december 1984, H. Geertsema), **3** L. phlaeas phlaeas (Nederland, Duurwoude, 1 september 1964), **4** L. dispar batavus (Nederland, Lemsterland, september 1969, F.A. Bink). De exemplaren bevinden zich in het Nationaal Natuurhisto- risch Museum Naturalis, Leiden.*

male genitalia, but the authors stress that the boundaries between the groups are not very sharp. The male genitalia of the South African species resemble the species of the *L. phlaeas* group and the *L. dispar* group in the relatively narrow valve with more or less parallel upper and underside and the long falces compared to the labides (figures 2-4). The species of the *L. thersamon* group also have long falces and the valve is also narrow, but it becomes narrower still towards the tip and then often more or less broadens again. The arms of the furca of the South African species are more like the long arms found in the *L. thersamon* group and in *L. dispar* than the shorter and more or less triangular arms in the *L. phlaeas* group. There is little resemblance in the male genitalia between the three other groups recognized by Bozano & Weidenhoffer (2001) and the South African species. The latter differ from all Palaearctic *Lycaena* species in the wings being more rounded. Although the genus *Lycaena* can be subdivided into a number of species groups on the basis of a combination of morphological characters, the number of characters is too low for a successful phylogenetic analysis. Therefore we tried to find the relationships with the help of DNA.

The African representatives

In the Afrotropical and neighbouring Arabian range of *L. phlaeas* the species is restricted to montane grasslands. Since these habitats are disjunctly distributed and, thus, the populations are more or less separated from each other, it is not surprising that local forms have developed. The populations of the south-western Arabian Peninsula (Saudi Arabia: Asir Mountains; Yemen) have been described as subspecies *shima* Gabriel, 1954. This subspecies is more similar to the East African populations than to the populations of the Middle East (Larsen 1984). The Ethiopian populations have been named subspecies *pseudophlaeas* Lucas, 1866. The form occurring in the high mountains around Lake Kivu (Kigezi District in South-west Uganda; East Zaïre; Rwanda) and east to the Ngara District in North-

west Tanzania (figure 5) has been described as subspecies *ethiopica* Poulton, 1922. D'Abrera (1980: 525) states that it occurs in the 'Ruwenzori Mts of Kigezi Dis-trict in south-western Uganda and adjoining areas of Zaïre' (copied by Ackery *et al.* 1995: 619). This is incorrect. The Kigezi District is far to the south of the Ruwenzori mountains. Copper butterflies are not known from Ruwenzori. Some authors (e.g. Stempffer 1967, Berger 1981) consider this form to be the same as the Ethiopian form, but others (e.g. D'Abrera 1980, Kielland 1990, Ackery *et al.* 1995) recognize it as a separate subspecies.

Whatever the exact status of these forms, *shima*, *pseudophlaeas* and *ethiopica* resemble the populations of the species in the Middle East and further north in the upperside of the hindwing being brown with an orange band (figure 6a, b). This is quite different from the form found from the highlands of Kenya through the Eastern Arc mountains in Tanzania to South Malawi (Gifford 1965, Kielland 1990, Larsen 1991, de Jong & Cong- don 1993, Ackery *et al.* 1995), described as subspecies *abbottii* Holland, 1892 (rarely correctly spelled, usually found as *abotti*, *abboti*, *abbotii* or *abbotti*), in which the upperside of the hind-wing is orange with black spots (figure 6c). The latter form is either conside-
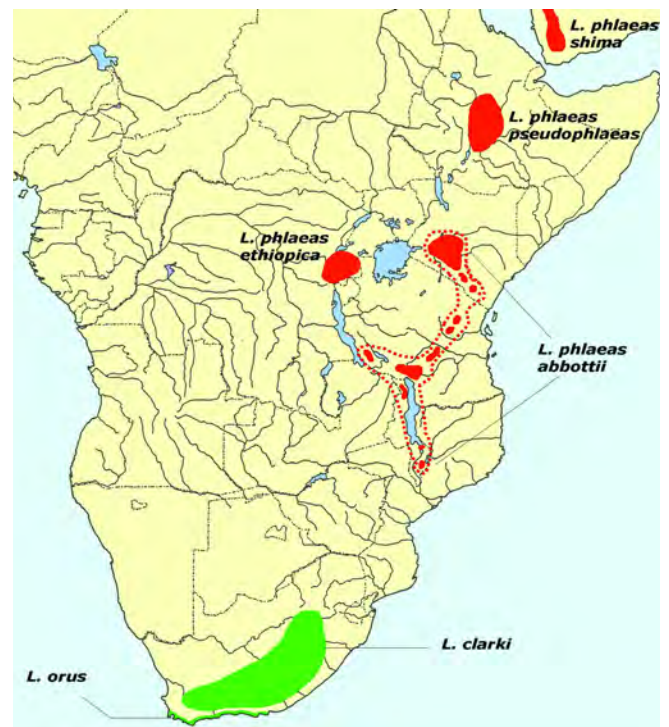

Figure 5. Representation of *Lycaena* in Africa. Illustration: R. de Jong
Verspreiding van Lycaena *in Afrika.*

red a subspecies of *L. phlaeas* or a separate species (Kielland 1990). Stempffer (1967: 264), who listed *abbottii* as a separate species, remarked that the genitalia resemble those of *phlaeas* so closely 'that it is permissible to wonder whether it is a true species or a geographical subspecies of *phlaeas*.' Larsen (1991: 213) was of the opinion that if *abbottii* is given species rank, the same would apply to *shima* and *pseudophlaeas*, and 'this would only serve to obscure the very real relationship of the Kenyan subspecies with the Palaearctic species.' This is only partly true. Larsen suggests that the three forms are all equally closely related to the Palaearctic populations, but that remains to be studied. It is well possible that *shima* and *pseudophlaeas* are more closely related to the Palaearctic populations than *abbottii*. The forms are widely separ- ated geographically and there is no proof of potential inter- breeding. Superficially *ab-bottii* is more different from *shima* and *pseudophlaeas* than *L. clarki* is from *L. orus* (see below), while the latter two are con-sidered separate species. Nomenclaturally all subspecies of a particular species have the same rank, but this has little to do with interrelationships.

The South African species *L. orus* (figure 6d) and *L. clarki* (figure 7) resemble *abbottii* in the upperside of the hindwing being orange with black dots. They can, however, easily be re-cognized by the more rounded wing shape and the generally reduced spotting. Fresh males have a violaceous lustre on the forewings, which is lacking in *abbottii* (and other forms of *L. phlaeas*). *Lycaena orus* is the southernmost one, occurring fr-om the Cape Peninsula to as far east as Port Elizabeth, in coastal districts and a little bit inland. *Lycaena clarki*, on the other hand, does not occur along the coast but is distributed from the West Cape to Kwazulu-Natal and Gauteng (South Transvaal). Although the two taxa differ only subtly in the un-derside of the hindwing as well as in the larva, the differences are said to be constant, indicating absence of gene flow between the two. Actual sympatry has not been recorded. The justification of the specific separation is not an issue in the pre-sent study.

Since *L. phlaeas* (s.l.) is the only other copper butterfly in Subsaharan Africa, it seems obvious that the South African taxa are more closely related to *L. phlaeas* than to any of the other *Lycaena* taxa. However, obviousness is not necessarily a feature of truth.

Analysis

The DNA-analysis was executed in the joint laboratory of the National Museum of Natural History Naturalis, IBL (University Leiden) and the National Herbarium - Leiden branch, The Netherlands, as well as in the laboratory of Prof. Naomi Pierce, Harvard University, Cambridge, Massachusetts, USA. In this con-tribution we focus on the African representatives. For technical details and some preliminary results of the DNA analysis we re-fer to Van Dorp (2004). See also the technical details in the box. For the present contribution we re-analyzed some of the data.
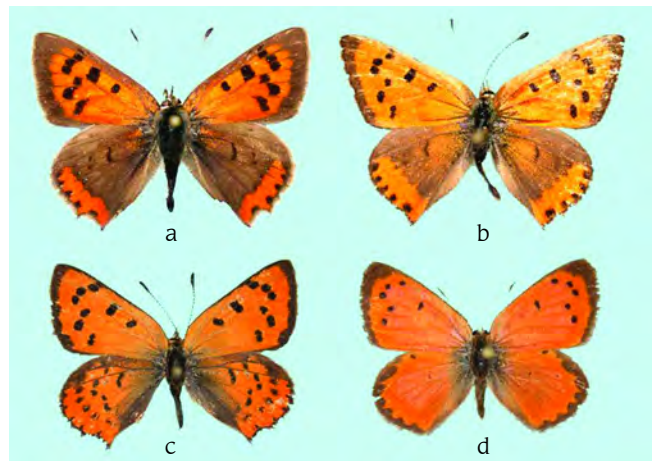

Figure 6. *Lycaena phlaeas* and *L. orus*. **a** *L. phlaeas phlaeas* (The Nether-lands, Roden, 27 July 1958, W.L. Blom), **b** *L. phlaeas shima* (Yemen, Jibla, 20 December 1974, K. Vegter), **c** *L. phlaeas abbottii* (Kenya, Kericho Dis-trict, Londiani, 2300 m, 25 October 1974, R. de Jong), **d** *L. orus* (South Africa, between Mamre and Jacob's Baai, 1 December 1926, Clarck). All specimens are males and are deposited in the collection of the National Museum of Natural History Naturalis, Leiden. Photo: R. de Jong *Lycaena phlaeas en* L. orus. *a* L. phlaeas phlaeas *(Nederland, Roden, 27 July 1958, W.L. Blom), b* L. phlaeas shima *(Yemen, Jibla, 20 December 1974, K. Vegter), c* L. phlaeas abbottii *(Kenia, Kericho District, Londiani, 2300 m, 25 Oktober 1974, R. de Jong), d* L. orus *(Zuid-Afrika, Westkaap, tussen Mamre en Jacobs Baai, 1 december 1926, Clarck). Alle exemplaren zijn mannetjes en bevinden zich in de collectie van het Nationaal Natuur-historisch Museum Naturalis, Leiden.*

Figure 7. *Lycaena clarki*, South Africa, Western Cape, Matroosberg, 21 January 2004. Photo: R. de Jong
Lycaena clarki, *Zuid-Afrika, Westkaap, Matroosberg, 21 januari 2004.*

Although in this paper we focus on the interrelationship of the South African taxa, we have included all species for which we had molecular data in the present analysis, since omitting an arbitrary part from the analysis may (and did) lead to inadvertent and incorrect results in the topology of the tree. Here we describe the analysis and results in general terms; details on materials and methods will be published in a more comprehensive paper on the genus.

Two mitochondrial genes were sequenced, COI and COII. We also sequenced the nuclear gene *wingless* for a smaller number of taxa, but the analyses of *wingless* gave spurious results and we decided to postpone analyses of *wingless* until more sequences would be available. The present analyses are based on COI and COII only. Of several species we used more than one sample, but only one sample per species has been included in the trees of figure 9. We analysed the data set for each gene separately and for COI and COII combined. In addition we translated the COI and COII sequences into amino acids and ran the analyses again. We included *Heliophorus kiana* and *Melanolycaena altimontana* (Lycaeninae: Heliophorini) in the ingroup since the sistergroup relationship of Heliophorini and Lycaenini needed further examination. We selected *Arhopala epimuta*, *Lucia limbaria* (both Theclinae) and *Acrodipsas brisbanensis* (Polyommatinae) as outgroups, since the relationship of the Lycaeninae seems to be closest with these two subfamilies (Wahlberg *et al.* 2005). The DNA sequences of the outgroup taxa were taken from GenBank. The data were analysed for Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI); see details in the box.

Results

Not surprisingly in view of their close morphological similarity, the South African taxa *L. orus* and *L. clarki* always emerged as sister taxa. The MP analysis of COI yielded sixteen equally parsimonious trees. The same analysis of COII gave a single tree. Combining the two genes yielded six trees. In addition we translated the nucleotide sequences into amino acids and ran the MP analyses again. They gave eighteen, 40 and three trees, respecti-

vely. Surprisingly, *all* trees agree in placing the South African taxa as sister group of the remainder of the Lycaeninae. This is surprising, since morphological characters did not suggest such an arrangement. If confirmed by analyses of additional genetic markers it will have drastic taxonomic consequences, as discussed below. The majority rule consensus tree of the amino acid analysis based on COI+COII is given in figure 9a. The ML analyses gave similar results with regard to the position of the South African taxa. Each of the analyses (for COI, COII and COI+COII) yielded a single tree and always the South African taxa appeared as sister to the remainder of the Lycaeninae. The ML-tree for COI+COII is presented in figure 9b. Finally, we subjected the same datasets to a Bayesian Inference. This again gave similar results for the South African taxa. The BI-tree for COI+COII is given in figure 9c.

While the results with regard to the interrelationship of the South African taxa are very consistent, the bootstrap support for their position on the tree in the MP analyses is under 50%. Also the probability (BI) of a sister group relationship between the South African taxa and the remainder of the subfamily (or the probability of the remainder of the subfamily being monophyletic) is relatively low (74%, see figure 9c). Yet, in view of the consistency of the results we think that the inclusion of more taxa and more markers will lead to higher support values.

Although falling outside the scope of the present paper, we like to draw attention to the recurrent grouping of the bulk of the North American taxa (excluding *L. cupreus* and *L. phlaeas*) with two Central Asiatic taxa (which even may be included among the North American taxa) and the South-east Asian/New Zealand group. We shall report on this grouping elsewhere.

Taxonomic consequences

The position of the South African coppers as sister to the remainder of the Lycaeninae comes as a surprise, as their morph-

Figure 8. *Rumex ecklonianus* (= *lanceolatus*), food plant of *Lycaena clarki*. South Africa, Western Cape, Matroosberg, 21 January 2004. Photo: R. de Jong
Rumex ecklonianus *(=* lanceolatus*), voedselplant van* Lycaena clarki. *Zuid-Afrika, Westkaap, Matroosberg, 21 januari 2004.*
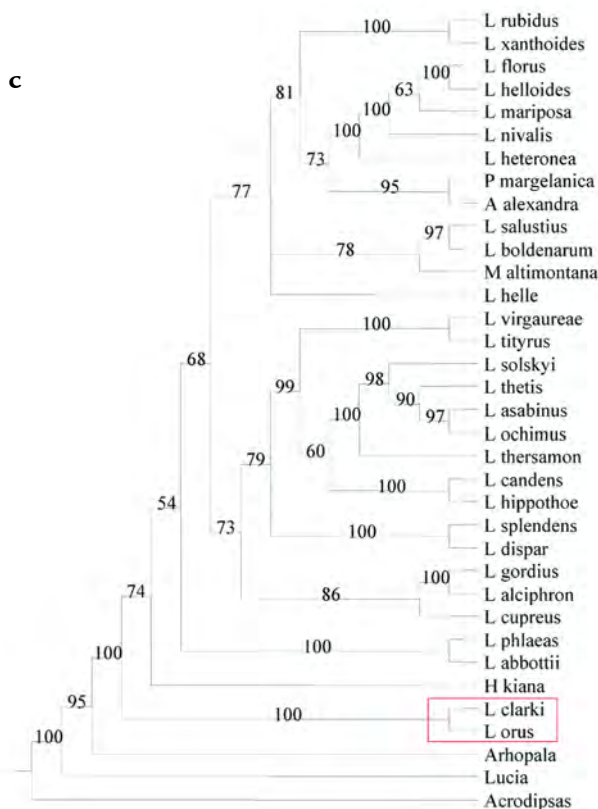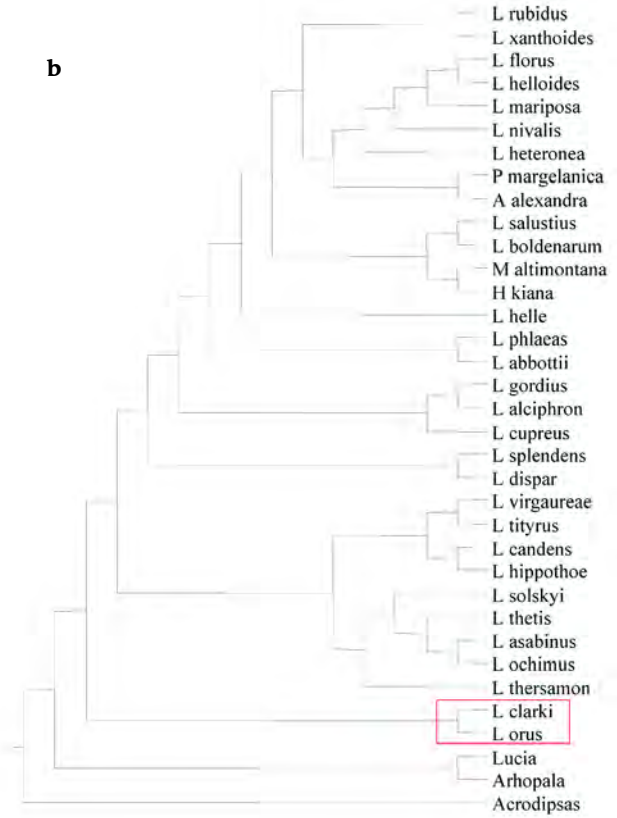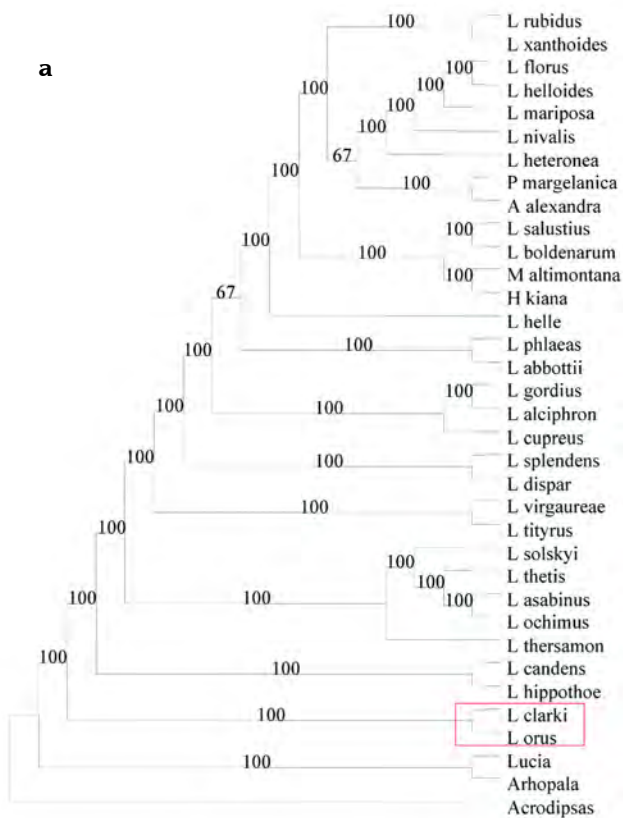
c

Figure 9. Phylogenetic analysis. **a** Maximum Parsimony, majority rule consensus tree based on the three equally parsimonious trees in an analysis of COI and COII combined and translated into amino acids; the numbers are the percentages of the trees in which the taxa to the right of the number are monophyletic, **b** Maximum Likelihood, single tree based on COI and COII combined, **c** Bayesian Inference, consensus tree based on COI and COII combined; the numbers are the probabilities of the respective clades being monophyletic.

*Fylogenetische analyse. **a** MP, majority rule consensus-boom, gebaseerd op sequenties van de genen COI en COII tezamen en vertaald in amino-zuren; de getallen geven het percentage aan van de bomen waarin de taxa rechts van de getallen als monofyletische groep voorkomen, **b** ML, de eni-ge boom uit een analyse van de genen COI en COII tezamen, **c** BI, consensus boom; de getallen geven met een waarschijnlijkheid in percen-tages aan dat de taxa rechts ervan een monofyletische groep vormen.*

ological similarity with the true coppers (Lycaenini) and dissimi-larity with the Heliophorini is so obvious. It not only suggests that Lycaenini and Heliophorini are not monophyletic, the South African species can no longer be included in *Lycaena* without making this genus paraphyletic, and also in the remainder of the genus *Lycaena* as currently understood, taxonomic changes must be executed to keep supraspecific taxa monophyletic, eit-her by lumping all remaining taxa into an umbrella genus *Lycaena* or by recognizing many smaller genera. Before taking such drastic steps we propose that first the interrelationships as suggested by the present study are confirmed by further genetic markers, particularly *wingless* and EF-1α.

Historical biogeography

As it turned out, the relatively close proximity and general resem-blance of the South African taxa and *L. phlaeas* were no keys to

their relationship. The distant relationship of the South African and New Zealand taxa does not come as a surprise. If the taxa in these areas were the remnants of a once widespread group of coppers across the southern continents, we would have to go back as far as *c*. 180 million years, when the southern supercontinent Gondwana started to disintegrate. All species of Lycaeninae, however, live on members of the plant family Polygonaceae (figure 8), except some apparently secondary shifts in North America. This plant family is supposed to be not older than 37 million year (Wikström *et al.* 2004) and, consequently, the subfamily cannot be older. Since the present distribution cannot be the result of the break-up of Gondwana, dispersal must have been the primary agent. Since the first divergence was between (taxa from) Africa (possibly their present restricted distribution is only a relic of a once much wider distribution) and the rest of the world, and since of the rest of the world Eurasia was much closer than any other part of the world, the first divergence seems to have taken place between Eurasian and African taxa. This agrees with the trees found in this study. It also implies that the diversification in South-east Asia (and down to New Zealand) and in North America is of a more recent date, which also agrees with the trees found here. We may even predict that the monotypic genus *Iophanus* from Guatemala, currently placed in the tribe Heliophorini and geographically much out-of-place, will have a much better understandable position as member of the clade that contains Central Asian, Southeast Asian-New Zealand and North American taxa.

Acknowledgements

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Summary

De verwantschap van de Afrikaanse vuurvlinders

De kleine vuurvlinder (*Lycaena phlaeas* Linnaeus, 1758) is dankzij een groot aanpassingsvermogen een van de wijdst verspreide dagvlinders ter wereld. Hij komt voor van de rand van de woestijn tot aan de Noordelijke IJszee en van zeeniveau tot 4500 m in het gebergte. Hij behoort tot de tribus Lycaenini (Lycaenidae - blauwtjes, subfamilie Lycaeninae) die met ongeveer 80 soorten voornamelijk op het noordelijk halfrond te vinden is. De kleine vuurvlinder is echter zuidwaarts verspreid tot in Malawi, terwijl twee andere vuurvlindersoorten endemisch zijn voor Zuid-Afrika. De tribus is niet vertegenwoordigd in Zuidoost-Azië, maar komt verrassenderwijs wel voor in Nieuw Zeeland met vier soorten. In Zuidoost-Azië leeft een andere tribus met twee genera, de Heliophorini, die bovendien nog een genus in Guatemala omvat. Om het ontstaan van deze eigenaardige verspreiding beter te kunnen begrijpen is kennis van de fylogenie van de betrokken taxa nodig. Deze is geanalyseerd aan de hand van DNA, omdat morfologische kenmerken niet toereikend bleken. In dit artikel wordt nader ingegaan op de Afrikaanse vertegenwoordigers.

In het zuidwestelijk deel van het Arabisch schiereiland en in oostelijk Afrika is de kleine vuurvlinder beperkt tot graslanden in het gebergte. Er worden verschillende ondersoorten onderscheiden: *shima* (zuidwestelijk Arabisch schiereiland), *pseudophlaeas* (Ethiopië), *ethiopica* in de wijde omgeving van het Kivumeer) en *abbottii* (hooglanden van Kenia, Tanzania en Malawi). Deze laatste, afwijkend van de andere ondersoorten in de grondkleur (oranje) op de bovenzijde van de achtervleugel, wordt soms als aparte soort beschouwd. De twee Zuid-Afrikaanse soorten (*L. orus* aan de kust, *L. clarki* in het binnenland) lijken zeer sterk op elkaar, maar verschillen duidelijk van de kleine vuurvlinder. Van alle vormen van de kleine vuurvlinder lijken ze het meest op ssp. *abbottii*.

Uit de analyses blijkt dat de Zuid-Afrikaanse soorten aan de basis van de ontwikkeling van de hele subfamilie (dus Lycaenini én Heliophorini) staan en de verscheidenheid die we nu zien op het noordelijk halfrond en in Zuidoost-Azië (en tot in Nieuw Zeeland) dus van later datum is. Aangezien alle soorten van de Lycaeninae op Polygonaceae (duizendknoopachtigen) leven (afgezien van enkele Noord-Amerikaanse soorten, die blijkbaar later zijn overgestapt) en deze plantenfamilie ongeveer 37 miljoen jaar geleden ontstond, is de hele ontwikkeling van de Lycaeninae ook niet ouder dan 37 miljoen jaar. Dit betekent dat de huidige verspreiding van de vuurvlinders tot stand is gekomen na het uiteendrijven van de continenten en het gevolg is van actieve uitbreiding van arealen tot over zeeën heen (om in Nieuw-Zeeland te komen).