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# "Blues and fashion": a case of polymorphism in females of Arhopala hercules herculina from West Papua, Indonesia (Lepidoptera: Lycaenidae)

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Abstract: Difficulties in separating males of A. herculina (STAUDINGER, 1888) and A. leo (DRUCE, 1894) made it desirable to investigate the relationship of both taxa using DNA analysis. Differences in mitochondrial COI gene and in the nuclear Ef-1 $\alpha$  gene sequences appear to be very low (0-0.15%), indicating that only one species with two female phenotypes is involved (blue female-form in "leo"; brown female-form in "herculina") and that leo must be regarded as a synonym of herculina. The status of A. herculina obscurata RIBBE, 1926 is revised as a synonym to A. hercules herculina STAUDINGER, 1888 and a female lectotype is designated (in SMT, Dresden). DNA-data and general distributional pattern, which shows sympatric occurrences for several other members of the hercules-group, suggest that - instead of species or subspecies - it is more likely that individual forms or phenotypes are involved.

### "Bläulinge und Mode": Ein Fall von Polymorphismus bei Weibchen von Arhopala hercules herculina aus West-Papua, Indonesien (Lepidoptera: Lycaenidae)

Zusammenfassung: Schwierigkeiten bei der morphologischen Unterscheidung der Männchen von A. herculina STAUDINGER (1888) und A. leo (DRUCE, 1894) machten es wünschenswert, die Verwandtschaftsverhältnisse mittels einer DNA-Analyse zu überprüfen. Unterschiede in den mitochondrialen COI- sowie die nukleären Ef-1a-Gensequenzen sind sehr gering (0-0,15 %), was darauf hinweist, daß nur eine Art mit zwei verschiedenen weiblichen Phänotypen existiert (blaue Weibchenform bei "leo" und braune Form bei "herculina"). A. leo wird daher als Synonym von herculina betrachtet. Der Status von A. herculina obscurata RIBBE, 1926 (als Synonym zu A. hercules herculina Staudin-GER, 1888) wird revidiert und ein Lectotypus Weibchen (in SMT, Dresden) designiert. Die DNA-Daten und allgemeinen Verbreitungsmuster von mehreren Taxa der hercules-Gruppe, die teils sympatrische Vorkommen zeigen, deuten darauf hin, daß es sich anstelle von Arten oder Unterarten eher um einen Komplex aus individuellen Varietäten oder Formen handelt.

# Introduction

Within the genus Arhopala BOISDUVAL, 1832, specimens of the hercules-group (sensu Evans 1957) belong – as their name suggests – to a group of especially striking and to, in general terms of lycaenids, large species, which have stirred the interest of collectors and researchers alike. Most of the species are not rare. It is therefore not surprising that numerous local races or subspecies were described, and the last comprehensive revision of Evans' lists not less that 9 subspecies just for A. hercules HEWITSON, 1862 (the nominotypical subspecies is only known from the Sulawesi area, Figs. 1–2), of which 5 appear to occur in West Papua. Separation of these local races is particularly difficult. This is especially the case with the mainland ssps. herculina and leo and, aside of thoughts concerning possible hybridisation, the question arises, if both could belong to the same species.

As far as West Papua is concerned, classification of *Arhopala hercules s.l.* based on Evans (1957) is as follows:

- A. hercules leo DRUCE, 1894 (TL Humboldt Bay),
- A. hercules herculina STAUDINGER, 1888 (TL Waigeo).

In addition to these "races" or subspecies, which are predominantly distributed in mainland West Papua, more island populations are known:

- A. hercules leontodamas (TOXOPEUS, 1930) (TL Misool).
- According to Evans (1957: 100), A. h. stymphelus FRUHSTORFER, 1914 co-occurs on Misool, thus being sympatric with *leontodamas* and questioning classification as subspecies. Evans (1957: 100) characterized it as "intermediate between *herculina* and *phalaereus*".
- A. hercules phalaereus FRUHSTORFER, 1914 (TL Japen).
- A. hercules sophilus FRUHSTORFER, 1914 (TL Obi). (According to EVANS, also occurring on mainland West New Guinea.)

PARSONS (1998) synonymized phalaereus and leontodamas with herculina, accepting only leo, herculina, sophilus and tyrannus C. & R. FELDER, 1865 as valid species. He retains the mainly Moluccan A. h. stymphelus as valid subspecies of hercules HEWITSON, 1862 and, as phenotypes from several localities are remarkably stable in their appearance, a separation may be justified. Aside from its type locality (Bacan), it is also recorded from Halmahera, Obi, Misool, Japen and Mioswaar (Figs. 3-4).

West Papuan specimens of *leo/herculina* are extremely variable concerning underside markings, reaching from almost unmarked to heavily marked specimens. Underside colour may range from a light mint green, sometimes almost white, to a dark brownish green with a pinkish hue. The dark median bands underly a similar variation. In extreme cases they vary from almost completely missing to very distinctive, broad, brownish bands. Even specimens from the same locality show this wide variation. Green tornal scaling is usually present.

This also raises the question why the wing markings of various taxa of the *hercules*-group are so variable, whereas in most of the other *Arhopala*-species variation of the underside markings is rather low and its pattern may be used to confidently separate different species? Reliable separation of sympatric *leo/herculina* males therefore seems impossible, which also questions the current classification, and genitalia structure is not of much help in this group. According to PARSONS, differentiation of QQ seems to be comparably easy, because of wing colouration. QQof *herculina* are supposed to have completely brown uppersides, sometimes with whitish or yellowish patches (Fig. 5), but without any blue, whereas *leo* QQ have basally blue wing uppersides (Figs. 6–8). This is a straightforward and simple way of determination, but leaves out the  $\sigma$  completely. Separation of  $\sigma$  specimens from localities where both "species" occur remains arbitrary or even impossible. Clearly, there is no general consent concerning the different views and to TENNENT & RAWLINS (2010: 13) classification appeared largely to be a "matter of interpretation".

It is known since a long time, that *leo* and *herculina* are sympatric and Evans assumed ecological cause or "invasions" from other areas to explain their co-occurrence. D'ABRERA (1977: 308) was the first one to ask with good reason, whether some races may be merely forms of the same species, rather than distinct subspecies. PARSONS (1998: 382) took a contrary position, proposing that "it is clear that the 10 supposed subspecies of *hercules* actually represent several closely related species". Following basically TOXOPEUS (1930), he changed the classification of EVANS and raised *leo* again to species rank, possibly because of its sympatric occurrence with *herculina* in Papua New Guinea (PNG): "A. *leo* is sympatric with its nearest relative, *herculina*, at Kiunga."

Even though acknowledging that both "species" are very closely related, PARSONS also says that they may be easily separated because in *herculina* specimens from PNG, the underside postmedian bands are much broader and may be more irregular than in *leo*, where they are "much straighter, narrower and sometimes vestigial". However, in his description of *leo* he mentions that the median band may be very distinct and only sometimes vestigial (PARSONS 1989: 383). This may be the case in PNG, but is certainly not so in West Papua, and especially female *leo*-specimens from Waigeo have very broad postmedian bands (Fig. 8).

Accordingly, colouration of the females remained as the only character to separate *leo* from *herculina*: *A. leo* has blue females, whereas the females of *herculina* are usually brownish.

Surprisingly, one possible cause has not been discussed in any of the previous works: polymorphism has never been considered, even though it is rather common and well-known from many lycaenid females, e.g. the Palaearctic *Polyommatus icarus* (ROTTEMBURG, 1775) or *Lysandra corydonius* (HERRICH-SCHÄFFER, 1804), which display a high variability depending on habitat conditions (VODOLAZHSKY & STRADOMSKY 2008, TALAVERA et al. 2013).

# Material and methods

Material used for DNA analysis:

QQ, Indonesia: 70 km NE Sorong, W. Papua, I. 2010, S. SCHRÖ-DER – voucher ILL\_243, ANGB KU189174 (COI), KU189185 (Ef-1α).

Arhopala hercules herculina f. leo: Q, Indonesia: Waigeo, x. 2009, S. Schröder – voucher ILL\_241, ANGB KU189186 KU189172 (COI), KU189183 (Ef-1α);

Q, ibidem – voucher ILL\_244, ANGB KU189175 (COI),
KU189186 (Ef-1α).

Arhopala hercules hercules: Q, Indonesia: Bantimurung, Sulawesi, IX. 2011, S. SCHRÖDER – voucher ILL\_242, ANGB KU189173 (COI), KU189184 (Ef-1α);

ď, Indonesia: Peleng, x. 2010, S. Schröder – voucher ILL\_247, ANGB KU189178 (COI), KU189189 (Ef-1α).

*Arhopala hercules* "Aru": ♂, Indonesia: Aru Isl., 1. 2010, S. Schröder – voucher ILL\_246, ANGB KU189177 (COI), KU189188 (Ef-1α);

𝔅, Indonesia: Trangan Isl, Aru Arch., v. 2010, S. Schröder − voucher ILL\_250, ANGB KU189179 (COI), KU189190 (Ef-1α);

2, ibidem – voucher ILL\_251, ANGB KU189180 (COI).

Arhopala hercules tyrannus: ♂, Indonesia: Tobelo, Halmahera, x. 2009, S. Schröder – voucher ILL\_245, ANGB KU189176 (COI), KU189187 (Ef-1α).

Arhopala pseudocentaurus:  $\mathcal{J}$ , Indonesia: Sumbawa, IX. 2014, B. DE GROOF – VOUCHER ILL\_248, ANGB KU189181 (COI), KU189191 (Ef-1 $\alpha$ ).

We sequenced the 5' (barcode) section of the mitochondrial gene Cytochrome-c Oxidase I (COI) and the nuclear Elongation Factor 1-alpha (Ef-1 $\alpha$ ). We used the following PCR primer pairs: forward, 5'- GGT CAA CAA ATC ATA AAG ATA TTG G-3' with reverse, 5'-TTG CTC CAG CTA ATA CAG GTA A-3' and reserve reverse 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' were used to amplify COI. Ef-1 $\alpha$  was amplified with forward, 5'- TGA AGG CCG AAC GTG AAC GTG G -3' and reverse, 5'- GCC ACC CCT TGA ACC AGG GCA T -3'.

The following cycling protocols were used: an initial 4 min denaturation at 94°C and 40 cycles of 40 s denaturation at 94°C, 40 s annealing at 53°C and 40 s extension at 72°C. Amplified fragments were separated using an automated sequencing machine ("Applied Biosystems 3500"). The analysis of primary nucleotide sequences was made with the help of the application BioEdit Sequence Alignment Editor, version 7.0.5.3 (HALL 1999). Cladograms built using MEGA6 (TAMURA et al. 2013) method Minimum-Evolution.

Arhopala pseudocentaurus (DOUBLEDAY, 1847) was used as an outgroup to root the tree.

Arhopala hercules herculina: Q, Indonesia: Sorong, W. Papua, VIII. 2013, S. Schröder – voucher ILL\_240, ANGB KU189171 (COI), KU189182 (Ef-1 $\alpha$ );

Figs. 1–14: Arhopala, museum specimens; a = uppersides, b = undersides of the same specimen. Specimens not to the same scale and smaller than original; original wingspan (where measured) see in legend. Scale bar (where present) = 1 cm. - Figs. 1-2: A. hercules hercules. Fig. 1: ♂, Peleng Is., Sulawesi (57 mm wingspan). Fig. 2: ♀, Bantimurung, Sulawesi (58 mm). - Figs. 3-4: A. h. "stymphelus". Fig. 3: Q, Japen, West Papua [KSP 10809]. Fig. 4: Q, Mioswaar Is., West Papua [KSP 62688] (54 mm). – **Fig. 5:** *A. h. herculina*. ♀, Sorong, West Papua (52 mm). **Figs. 6–8:** *A. herculina* "f. *leo*". **Fig. 6:** ♀, Kaimana, West Papua (55 mm). Fig. 7: Q, Timika, West Papua (49 mm). Fig. 8: Q, Waigeo Is., West Papua (56 mm). - Fig. 9: A. tyrannus sophilus, HT 3, Obi Is., North Maluku [BMNH #(V)1113 ex. FRUHSTORFER Coll.; © Trustees of the Natural History Museum] (56 mm). – Fig. 10: A. hercules obscurata, LT ♀, "Ansus", Japen Is., West Papua [SMTD, #02885] (54 mm). - Fig. 11: A. hercules tyrannus, ♂, Halmahera, Maluku (56 mm). – Figs. 12–13: A. hercules herculina. Fig. 12: 3, Terangan Isl., Aru, Maluku (53 mm). Fig. 13: 2, Aru, Maluku (52 mm). – Fig. 14: A. hercules herculina, ♀, Japen Is., West Papua. – All specimens in CSSK, except "KSP", now in UNCEN and BMNH.



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Text-Fig. 1: ME-cladogram based on the Minimum Evolution method of analysis of distances for COI DNA sequences of Arhopala hercules specimens.



Text-Fig. 2: ME-cladogram based on the Minimum Evolution method of analysis of distances for Ef-1 $\alpha$  DNA sequences of Arhopala hercules specimens.

### Abbreviations

- ANGB Accession number GenBank.
- BMNH The Natural History Museum, London (formerly British Museum (Natural History)), U.K.
- CSSK Coll. S. Schröder, Köln, Germany.
- UNCEN Coll. Cenderawasih University (Universitas Cenderawasih), Waena, Papua New Guinea.
- SMTD Senckenberg-Museum für Tierkunde, Dresden, Germany.
- ZSM Zoologische Staatssammlung, München (Munich), Germany.

# Results

To provide evidence for the idea of polymorphism, DNA sequence of several specimens, including blue ("*leo*") and brown ("*herculina*") females was analysed. Additional samples from Sulawesi (*hercules s.str.*), Waigeo, Aru and Halmaheira ("*tyrannus*") were also included. Analysis clearly shows that DNA sequences of all specimens are very similar, with a maximum Ef-1 $\alpha$  difference of 0.4%, and a maximum COI-difference about of 1.3% in *A. hercules*, which indicates that only one species is involved in the specimens investigated.

Because of the slight differences between *leo* and *herculina* it is most likely that both are not more than different varieties within the same subspecies. *A. hercules* from Sulawesi as well as the *hercules* form occurring on Aru appear as separate taxa. Based on the very little differences in DNA sequences it is also unlikely that *tyrannus* belongs to a separate species but is merely a subspecies or form of *hercules*.

Based on the DNA data, classification of West Papuan *hercules* is revised and taxa are arranged in the following way, which basically reflects the historical classification of Evans:

#### Arhopala hercules (HEWITSON, 1862)

hercules hercules (HEWITSON, 1862) [Sulawesi] hercules herculina STAUDINGER, 1888 = leo DRUCE, 1894 syn. n. = obscurata RIBBE, 1926 syn. n. [Japen] ?hercules "Aru form" hercules tyrannus C. & R. FELDER, 1865 [Bacan, Halmahera, Morotai, Kasiruta] (? = sophilus FRUHSTORFER, 1914)

Subspecific status of *tyrannus* remains speculative and additional information is needed to clear its relationship.

It is known to occur sympatrically with *h. stymphelus and h. herculina* on Halmahera and Bacan and there are a few isolated records from mainland New Guinea (PARSONS 1998: 383). Maybe all these taxa have to be considered just as forms in the *hercules*-complex, which displays a high phenotypic plasticity.

According to Evans (1957: 100), *A. obscurata* is a synonym of *A. sophilus* (Fig. 9), which is regarded as valid species by PARSONS, however, without explaining his classification. This synonymy has to be rejected, because *obscurata* and *sophilus* do not appear very close. As RIBBE (1926) has already mentioned in his description: "*Hercules* von Neu-Guinea, West-Küste, haben keine metallische Färbung an den Anallappen, auch sind die Unterseiten wie mit Milch übergossen. Die ganz dunklen Weiber könnten den Namen *obscurata* führen." FRUHS-TORFER (1914: 156) describes the upperside wing colour of females of *sophilus* as brown, sometimes with traces of blue in the discal area.

Furthermore, a possible Q obscurata-syntype of the Röber collection in Dresden (Fig. 10) from Ansus (Japen) shows the characters mentioned in Röber's description very well: it has a very light coloured underside with a milky tint and also lacks tornal green hindwing scales. In a list of species containing the taxa described by RIBBE in 1926, DRAESEKE (1926) made clear that all the specimens listed were marked as "Original" in the Dresden collection. It is not known if additional specimens were collected; but DREASEKE gave the individual numbers for each taxon and in the case of *obscurata* only  $1 \, Q$  is mentioned. No further material is known from the ZSM. This Dresden Qis herewith designated as lectotype of obscurata RIBBE, 1926 (in SMTD; specimen ID: 02885; "A. B. MEYER, 1873, Ansus"). If underside colouration is of any use in this group at all, sophilus differs strongly from obscurata in having a brown wing underside colour with very prominent tornal green scales. In general appearance it is much closer to tyrannus than to obscurata and Toxopeus (1930: 168) had already regarded sophilus as a subspecies of tyrannus. Accordingly, obscurata may be a synonym of herculina but not of sophilus.

Along with A. h. tyrannus (with brown wing undersides, Fig. 11) a second phenotype (A. hercules stymphelus) with dark moss-green undersides occurs sympatrically on Halmahera. This taxon closely resembles A. hercules hercules from Maluku (Fig. 3) and these records may represent the missing step-stones in its distribution towards Papua. So far, two records from PNG remain unconfirmed (TENNENT & RAWLINS 2010, PARSONS 1989).

Specimens from the Aru Islands are here tentatively assigned to *herculina* (Figs. 12–13), even though DNA-data suggest that they slightly differ from the mainland race. Females are completely brown and underside colour is variable; there are specimens with light green/ mint green as well as pink undersides. They are lacking tornal green scales.

At this time, systematics of *A. phalaereus* remains unsettled. Distribution includes Japen, Mioswar Isl. and "W. New Guinea" (EVANS 1957: 100). Females are uniformly dark brown on the upperside and it is likely that the taxon is a synonym of *A. herculina*, which was already proposed by PARSONS. Along with these brown females, typical blue *herculina* f. *leo* females are also flying on Japen (Fig. 14).

# Acknowledgements

Thanks to John TENNENT, who photographed the typespecimen of *A. tyrannus sophilus* (Photos © of the Trustees of the Natural History Museum), to Blanca HUERTAS (BMNH), Wolfgang A. Nässig (SMFL) for editing photos and text and Henk VAN MASTRIGT(†).

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Received: 23. x1. 2015

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Zeitschrift/Journal: Nachrichten des Entomologischen Vereins Apollo

Jahr/Year: 2016

Band/Volume: 37

Autor(en)/Author(s): Schröder Stefan, Stradomsky Boris V.

Artikel/Article: <u>"Blues and fashion": a case of polymorphism in females of Arhopala</u> <u>hercules herculina from West Papua, Indonesia (Lepidoptera: Lycaenidae) 73-77</u>