






The Albanian expeditions... and the discovery of a new cryptic species for science



24 febr 2024 - V.V.E. Werkgroep dagvlinders

Laurian Parmentier

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Overview

- Intro
 - De eerste expedities
 - Contact Unief Tirana
- Een nieuwe vondst binnen het genus *Agrodiaetus*
- De wetenschappelijke beschrijving
- Kadering en nieuwe inzichten
- Vervolg

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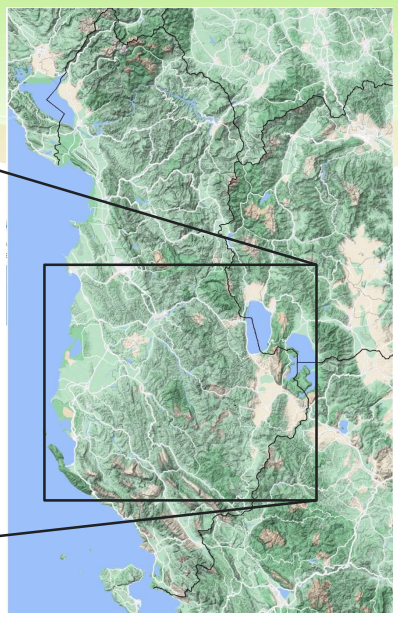


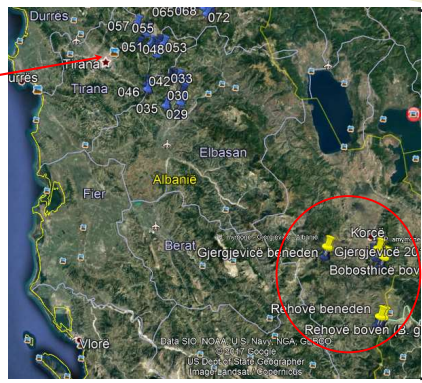
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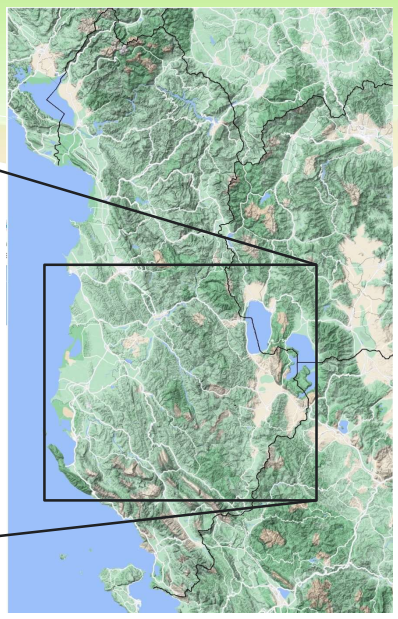
De eerste expedities 2015-2017

1. Zuid-oost Albanië





Tirana



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Albanië: de eerste expedities: 2015-2017
Eerste contact VVE met Universiteti i Tiranës, Fakulteti i Natyrës

Afspraak met wijlen Prof. Misja –
En Prof Papparisto
Overlopen interessante biotopen



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Albanië: de eerste expedities: 2015-2017

Boek Misja 2005

Eerste overzichtswerk met
Verspreidingskaartjes

Tot dan quasi ongeken
referentiewerk in
entomologische wereld



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Albanië: de eerste expedities: 2015-2017

Boek Misja 2005

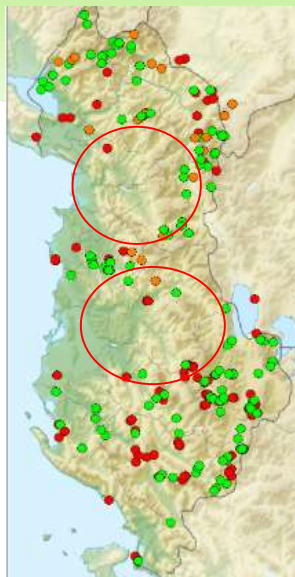
**Kaart met hyaten
Albanië,**

Vb. *M. jurtina*

Rood: observaties < 2018

Groen: > 2018

Cuvelier S., Parmentier L.,
Paparisto A. & Couckuyt J.
2018. Butterflies of Albania –
Fluturat e Shqipërisë. New
surveys, new species and a
new checklist (Lepidoptera:
Papilionoidea) S4:
distribution maps for all
species.



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Albanië: de eerste expedities: 2015-2017

Intussen: publicatie Vishnevskaya et al. (2016)

SPECIES | ACCEPTED

Polyommatus timfristosi Lukhtanov, Vishnevskaya & Shapoval, 2016

Published in: Vishnevskaya, Maria S., Alsu F. Saifiddinova & Vladimir A. Lukhtanov. 2016. Karyosystematics and molecular taxonomy of the anomalous blue butterflies (Lepidoptera, Lycaenidae) from the Balkan Peninsula. Comparative Cytogenetics 10(5): 1-85. source: ZooBank

OVERVIEW
TREATMENT
METRICS
REFERENCE TAXON
OCCURRENCE

1 OCCURRENCE WITH IMAGES



1 GEOREFERENCED RECORD

P. aroaniensis

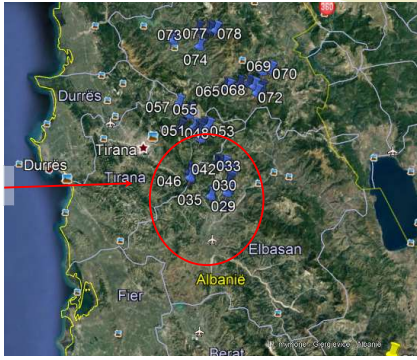
This taxon was first described by Brown (1976a) as a subspecies of *P. alcestis* and two years later was raised to species rank (Brown and Coutsis 1978). Despite its similarity to other taxa of the brown complex, especially with *P. humedasa*, *P. orphicus orphicus* and *P. o. eleniae*, it differs by its karyotype and COI barcodes... However, there are numerous identification problems associated with *P. aroaniensis* because several populations from Central and Northern Greece, as well as from other countries of the Balkan Peninsula were identified as *P. aroaniensis* (see the section Distribution areas below), but their karyotypes were not studied. In our work, we discovered that two of these populations (from Timfristos Mt and Parnassos Mt) represented a previously unrecognized species. Below we name it and provide its formal description.

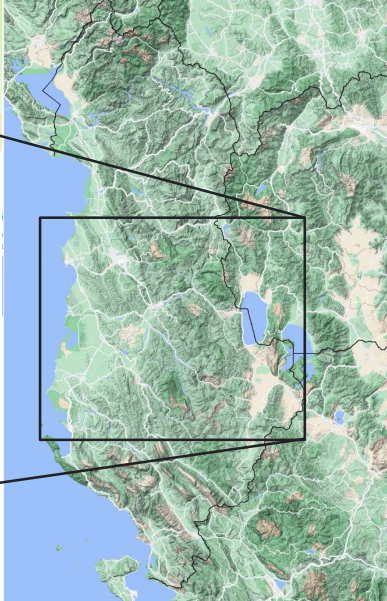


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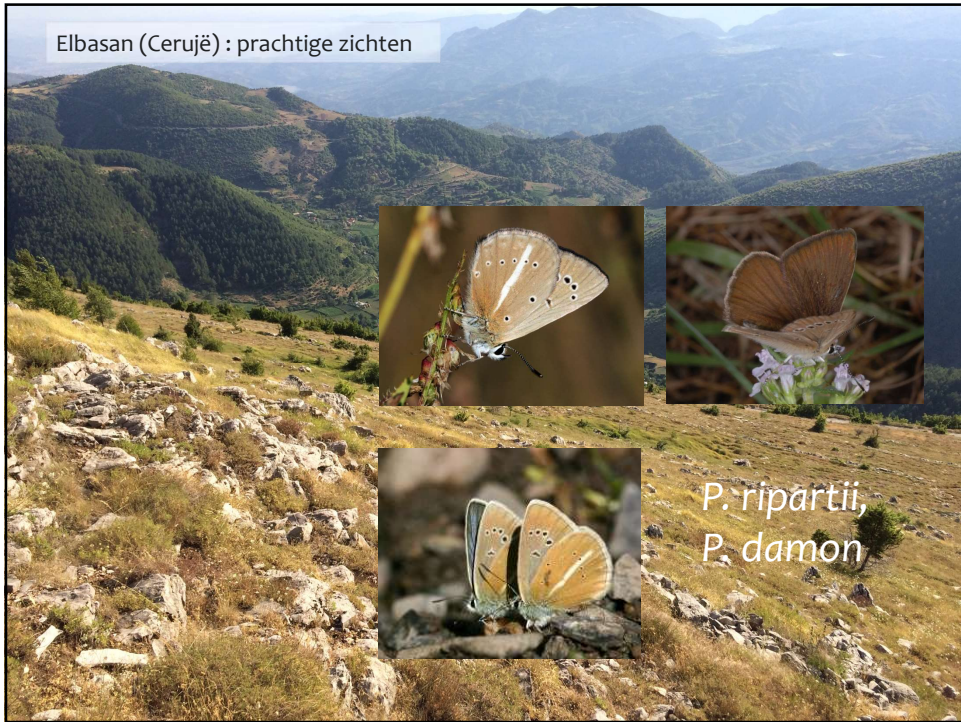
De eerste expedities 2015-2017

2. Midden Albanië





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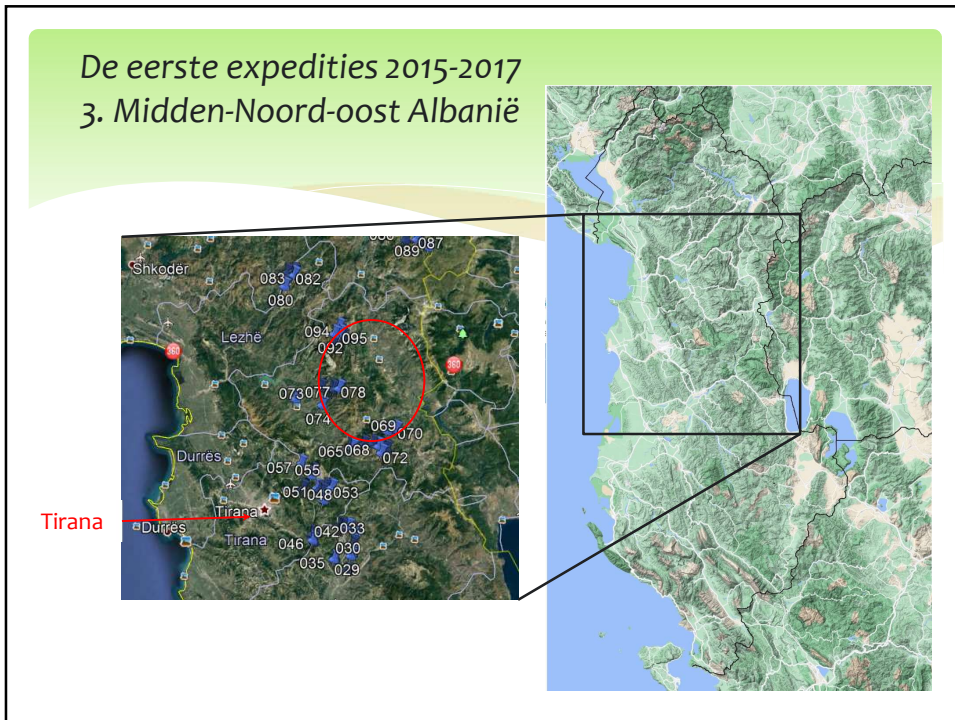
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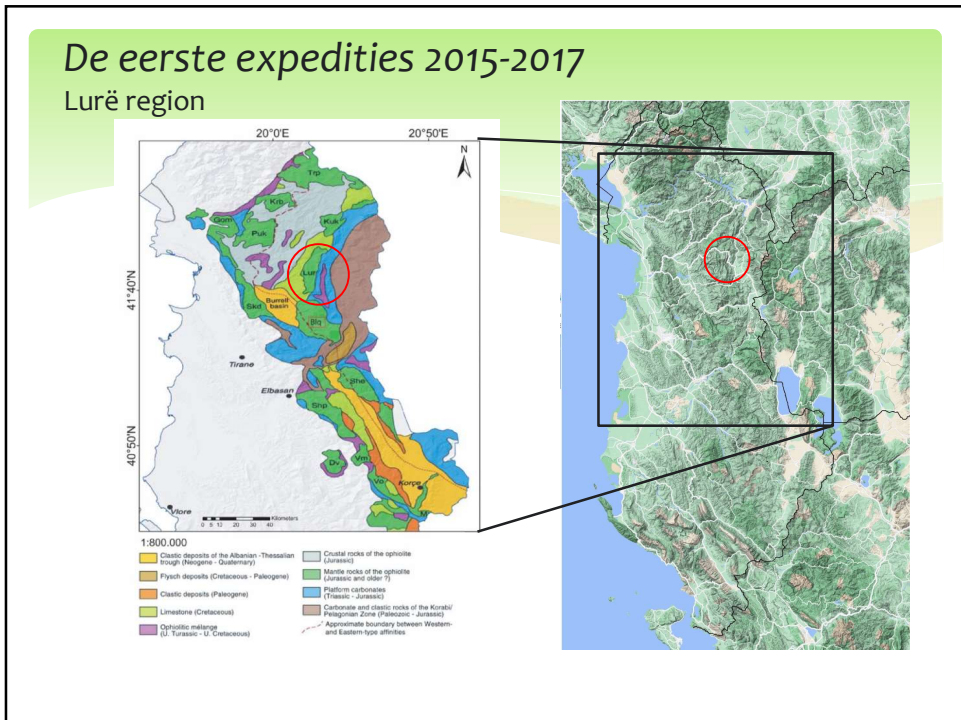
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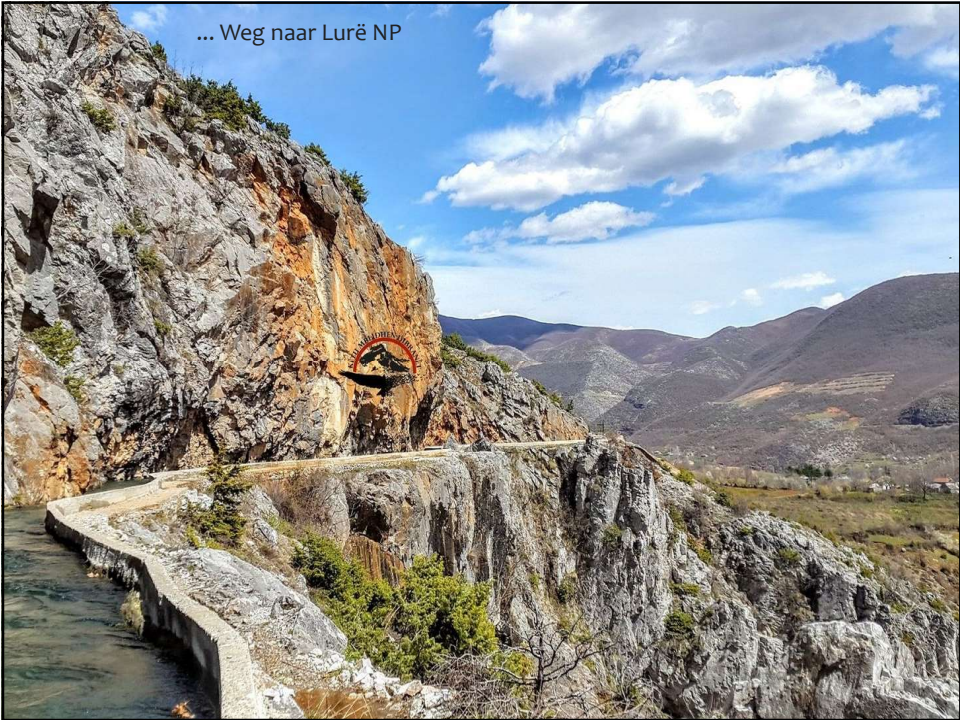
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1) Morfologische kenmerken

* Witstreepblauwtje
+ oranjetipje
=> 2 soorten



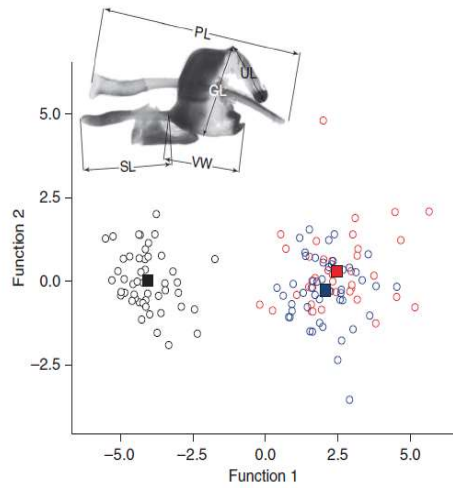

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2) Genitaliën

Leptidea spec. studie

- L. sinapis
- L. reali/juvernica ?

Figure 4 | Discriminant analysis based on male genitalia morphometry.
L. sinapis (black) is identifiable based on male genitalia, but there is broad overlap between *L. reali* (red) and *L. juvernica* stat. nov. (blue). Circles represent individuals and squares represent centroids for each species. Elements of the male genitalia measured were PL, SL, VW, GL and UL. The discriminant variables were PL and SL for function 1 and SL and VW for function 2. The upper left corner image indicates the variables measured for *Leptidea* male genitalia.

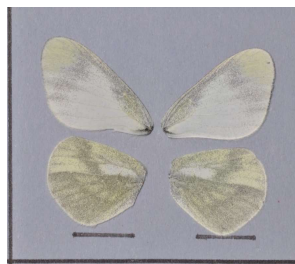


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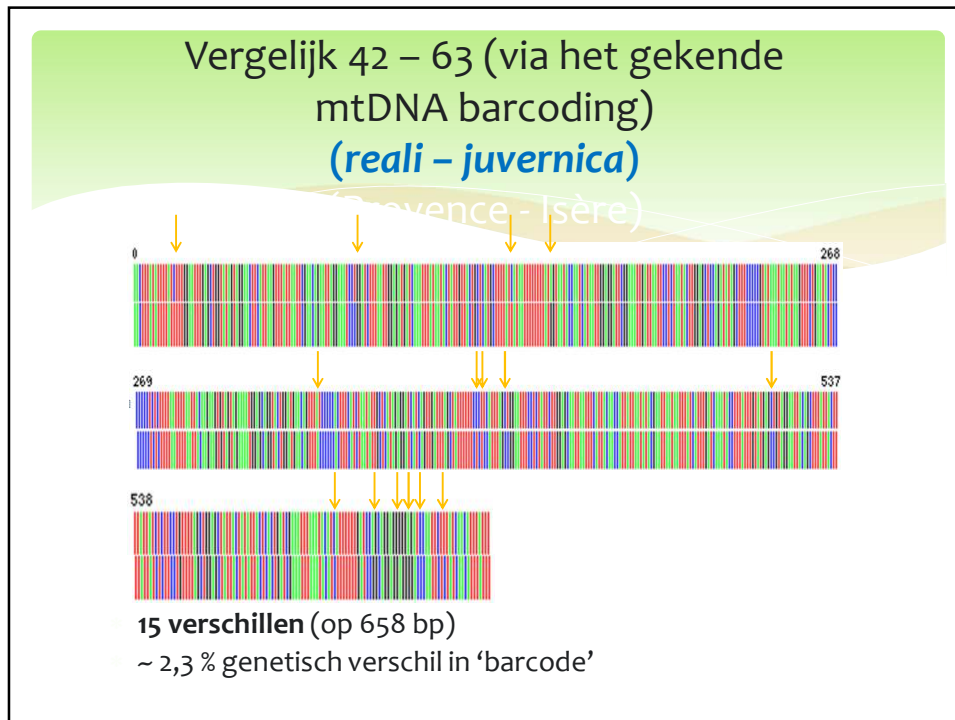
Boswitjes-onderzoek

* 42 : L. ??? (Provence)

63 : L. ??? (Isère)



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3 (a.) mt-DNA barcoding

Per soort ook nog variatie van mtDNA binnen de soort
Haplotypes

- => Interspecifiek (tussen soorten) ?
- => Intraspecifiek (binnen soorten) ?

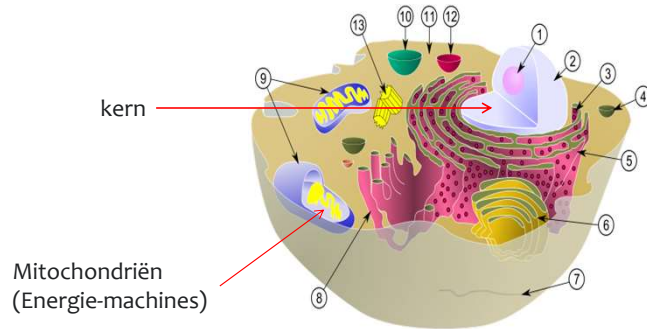
IGV : "Atlas of mitochondrial diversity of Western Palearctic butterflies"

- => mt DNA bezorgt ons informatie over de evolutie van en binnen soorten en soortvorming.

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3 (b.) nuclear barcoding/ karyotyping

Per soort ook variatie van kern DNA:
 Echte soorten zijn ook in hun kernDNA verschillend
 ~ genoom /full genome sequencing

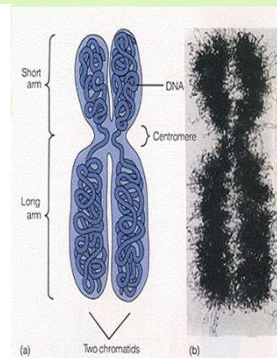
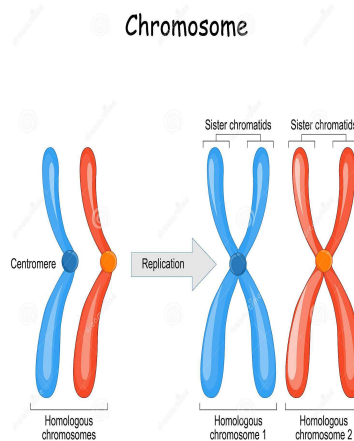


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kern DNA & Chromosomen

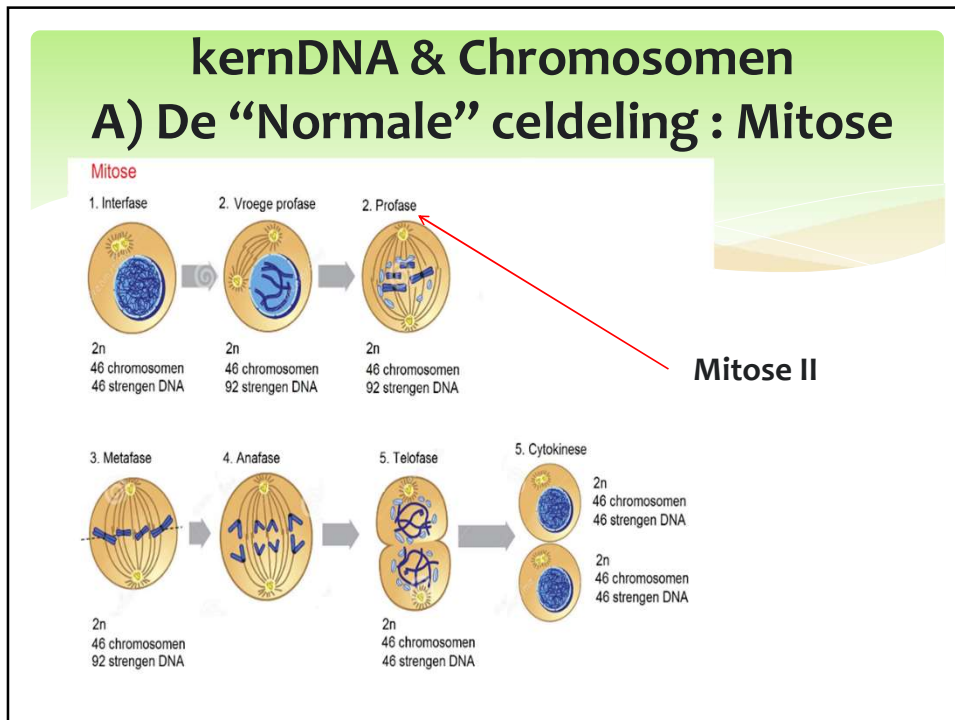
Chromosoom

- Deel 1) kopiëren/verdubbelen :
- Bivalent chromosoom
- Bestaan uit 2 dezelfde (zuster) chromatiden

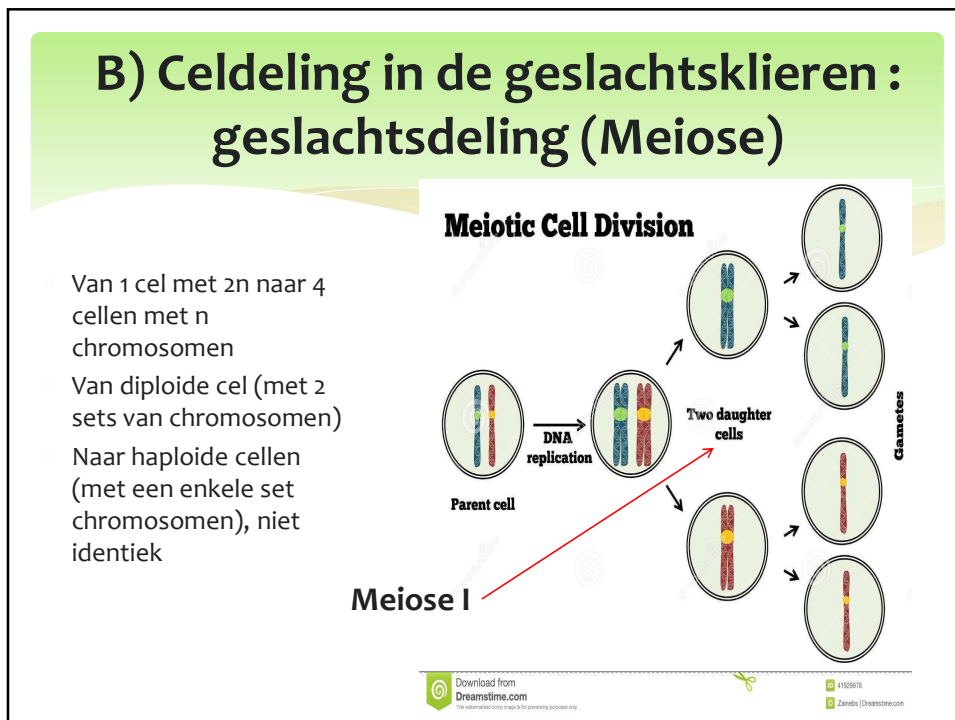


Een chromosoom, maximaal gecondenseerd DNA, bestaat uit twee exacte kopieën van een DNA-streng. Deze kopieën worden chromatide genoemd. Een chromosoom bevat dus twee strengen DNA.

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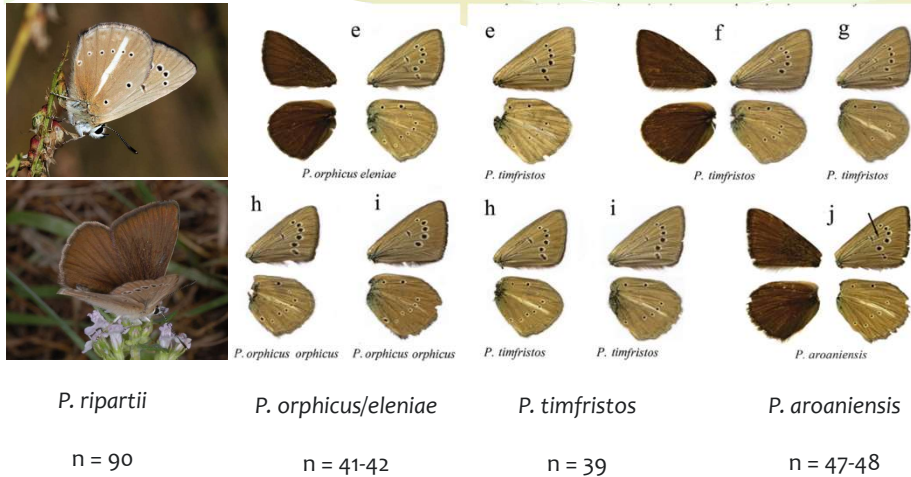


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Toegepast op enkele gekende Balkan *Agrodiaetus* soorten



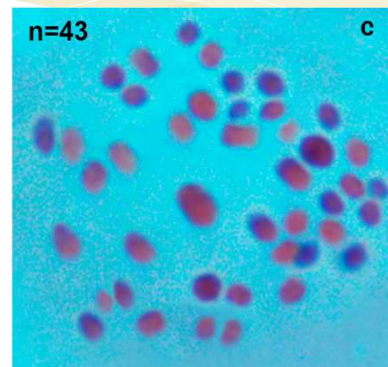
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Onderzoek specimen Lurë: wat is het?

Nu kunnen we aan de slag

1. Uiterlijk: veel donkerder... geen wit streepje
2. Mt DNA (barcode): zeer verschillend! =>?
3. Karyotype: ongekend (n = 43)

Conclusie:
iets nieuws...
maar meer stalen nodig voor uitsluitel !?!



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- *Extra samples...*
- De wetenschappelijke beschrijving
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Lurë: waar zijn de bruine *Agrodiaetus*?








Parmentier L., Vila R. & Lukhtanov V. 2022. Integrative analysis reveals cryptic speciation linked to habitat differentiation within Albanian populations of the anomalous blues (Lepidoptera, Lycaenidae, *Polyommatus* Latreille, 1804). – *Comparative Cytogenetics* 16(4): 211-242.2022

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1. Morfologisch

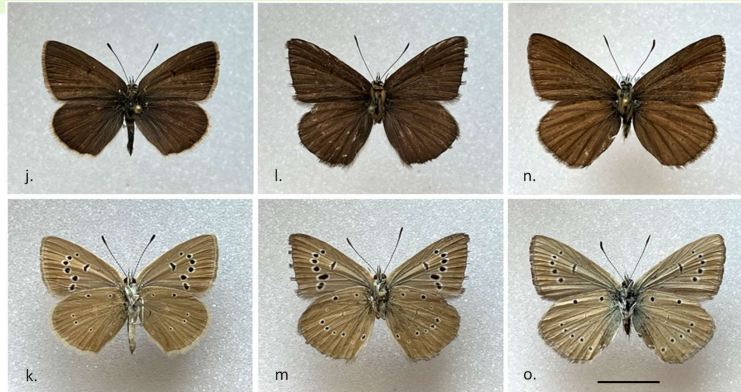
Vergelijken van nieuwe stalen met dicht aanleunende soorten op uiterlijk

			<i>P. lurae</i> M
			
			

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1. Morfologisch

Vergelijken van nieuwe stalen met dicht aanleunende soorten op uiterlijk



P. lurae
F

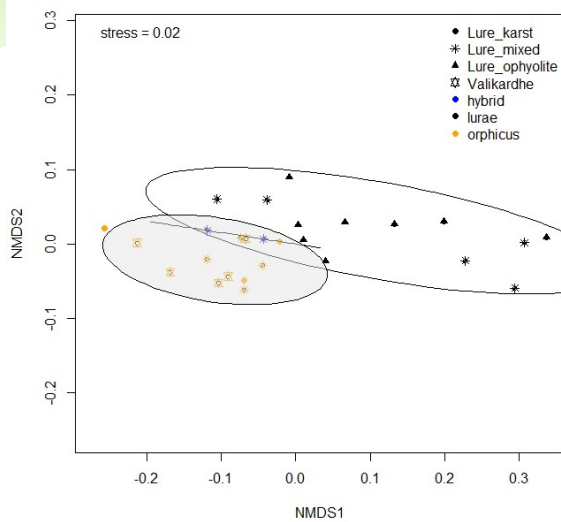
P. lurae
M

P. ripartii
M

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1. Morfologisch

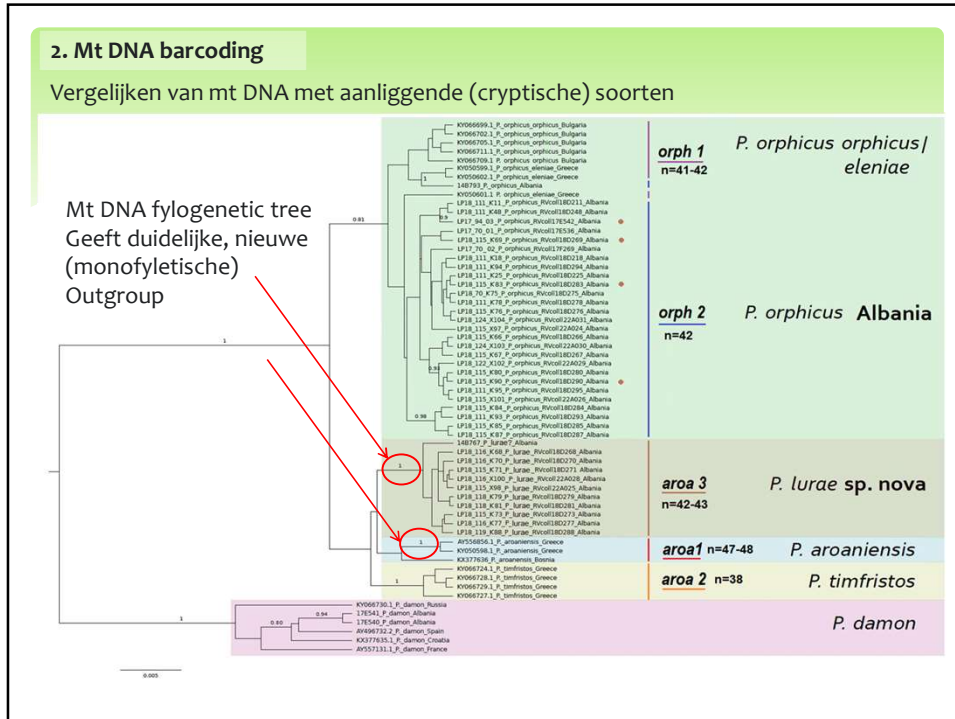
Vergelijken van nieuwe stalen met dicht aanleunende soorten op uiterlijk



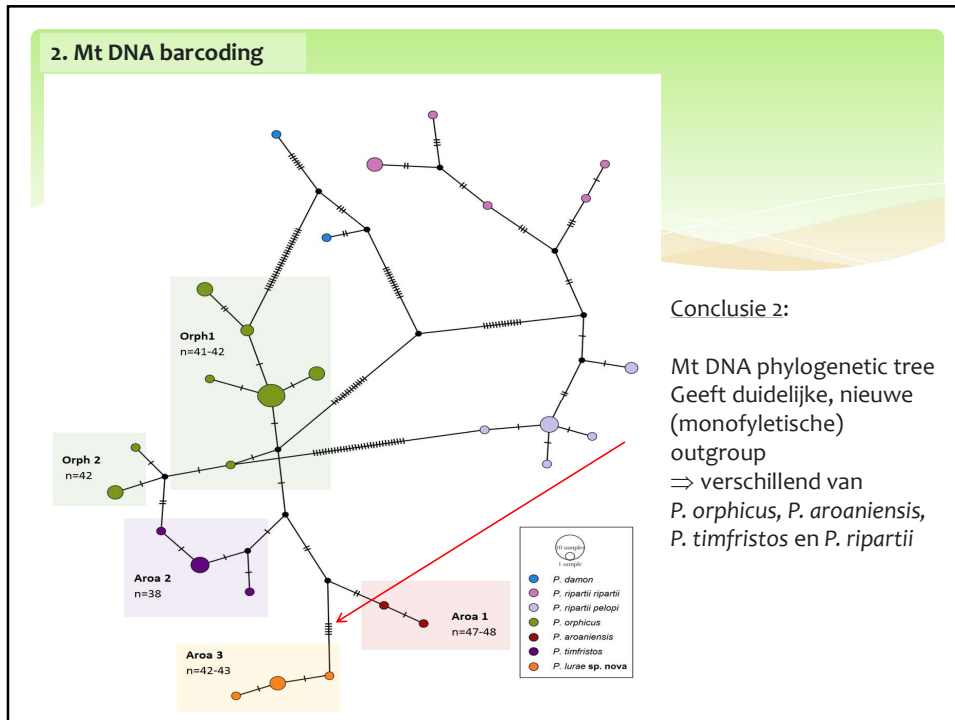
Conclusie 1:
Mbv. RGB metingen van
vleugels
(hoek => max. reflectie):
P. lurae significant *
verschillend van *P. orphicus*

* $P < 0.05$

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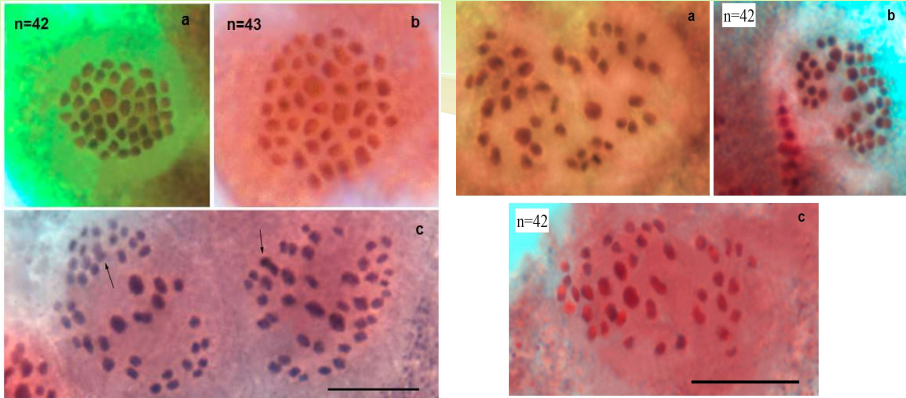
Conclusie 2:

Mt DNA phylogenetic tree
Geeft duidelijke, nieuwe (monofyletische) outgroup
⇒ verschillend van *P. orphicus*, *P. aroaniensis*, *P. timfristos* en *P. ripartii*

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3. Karyotyping

Vergelijken van kern DNA



P. lurae sp. nova
n = 42-43

P. Orphicus (Albanië)
n = 42

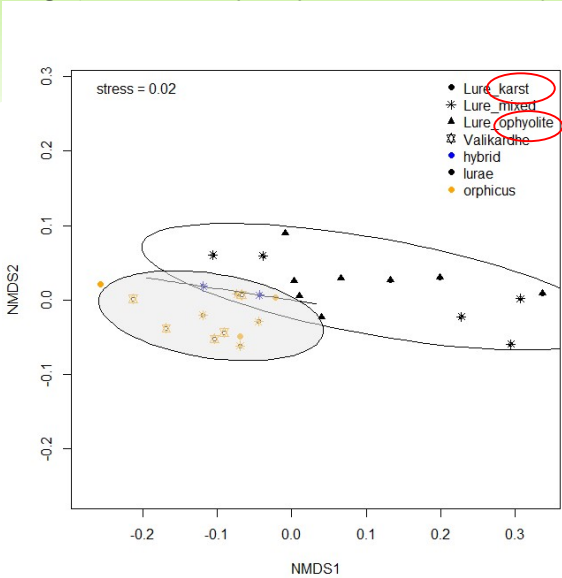
Conclusie 3:

Kernonderzoek geeft nieuwe karyotype, verschillend van *P. orphicus* (n =41-42), *aroaniensis* (n=47-48), *P. timfristos* (n = 39) en *P. ripartii* (n = 90)

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4. Biogeografisch – Reproductieve isolatie?

Vergelijken van biotoop vindplaatsen *P. lurae* vs. *P. orphicus*



Conclusie 4:
Reproductieve isolatie ifv. biotoop (ophioliet vs karst)

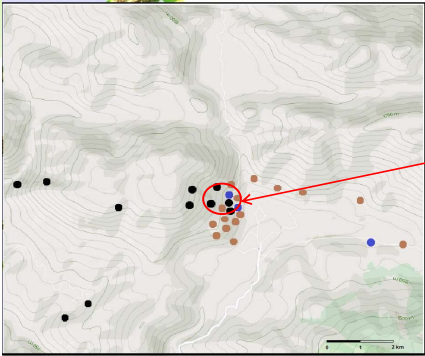
P. lurae significant *
verschillend van *P. orphicus*

* $P < 0.05$

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Opmerking: wel hybridisatie tussen *P. lurae* en *P. orphicus*

Vastgesteld op enkele (4 exemplaren)



Contactzone

Is dat een probleem?


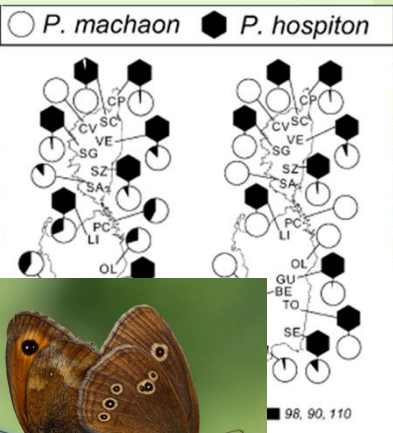
Neen,
Indien er reproductieve isolatie is

Moelijkere term, cytologisch gezien:
'reinforcement na prezygotic isolation'
⇒ Hybridisatie tussen verder geëvolueerde lineages
⇒ Verdere split van de lineages
(Minder 'fitted' ~ basisprincipe Darwin)

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Recap: Hybridisatie tussen soorten: is dit een unicum?

- Presentatie Laurian 2 febr. 2012 VVE WG DV
- Artikel BLK Laurian *

○ *P. machaon* ● *P. hospiton*

- Fotowedstrijd Flieflotter Laurian



- Steeds meer voorbeelden tussen 'duidelijke soorten'...

* Parmentier, L. Hybridisatie tussen *Papilio machaon* LINNAEUS en *Papilio hospiton* GENÉ : veldervaringen en kweek "in labo". BLK-CLB 2012

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Hybridisatie: dus geen soort??
 Geen morfologisch verschil: dus alles zelfde als één 'superspecies'??

Lumpers of splitters... what's in a name? Klein artikel Cuvelier (2023), Lépidoptères



aroaniensis-morph



Polyommatus lurae

Toekomst van fylogenie = Integrative aanpak
Zeker voor cryptische soorten(groepen)

Fig. 7b. Map of barcoded *P. lurae*.

<p>* in the Monographs treated as</p> <p>** in the Monographs treated as</p>	<p><i>Polyommatus (aroiensis) lurae</i></p> <p><i>Polyommatus (aroiensis) orphicus</i></p>	<p>Status: needs more study</p> <p>Status: needs more study</p>
------------------------------------------------------------------------------	--------------------------------------------------------------------------------------------	-----------------------------------------------------------------

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Recente reflectie in PNAS artikel

Should All Lineages Be Species?

While evolutionary biologists tend to leave phylogeographic lineages unnamed, taxonomists tend to oversplit them into multiple species (20, 21). In principle, the scientific community largely agrees that species correspond to diverging populations that eventually become biologically isolated (the endpoint of speciation) (22). However, disagreements remain around how to delimit them in practice, i.e., whether to put the emphasis on divergence and evolutionary independence (the lineage species concept, ref. 23), or the process that leads to it, i.e., reproductive isolation (the biological species concept, ref. 24). Diagnosability, i.e., setting up applicable criteria of identification and delimitation, is central to the practice of taxonomy, and because reproductive isolation is hard to assess, **most candidate species are preferentially characterized based on their molecular or phenotypic divergence, combining genetic, morphological, behavioral, and ecological evidence (e.g., integrative taxonomy, ref. 25).** The overreliance on molecular analyses to define species has progressively led to consider any diagnosable phylogeographic lineage as a "species under construction", and by extension, to their recognition as species (26). This shift of paradigm now reaches its climax, with the species rank being frequently claimed to be the only relevant terminal rank in alpha-taxonomy (27).

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Comparative Cytogenetics

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Research Article Comparative Cytogenetics 16(4): 211-242
https://doi.org/10.3897/CompCytogen.v16i4.90558 (15 Nov 2022)

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Integrative analysis reveals cryptic speciation linked to habitat differentiation within Albanian populations of the anomalous blues (Lepidoptera, Lycaenidae, *Polyommatus* Latreille,

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known to be represented by three closely related allopatric species, differentiated by their chromosome numbers (n) and mitochondrial (mt) DNA. These are *P. aroaniensis* sensu stricto (Southern Greece, Peloponnese, n=47-48; mt haplogroup *aroa1*), *P. timfristos* Lukhtanov, Vishnevskaya et Shapoval, 2016 (Central Greece, Attika, n=38, *aroa2*) and *P. orphicus* Kolev, 2005 (North-Eastern Greece, Southern Bulgaria, n=41-42, *orph1*).

Based on an analysis of chromosomal, molecular and morphological markers, we demonstrate that a fourth taxon of this species complex exists in Albania. This taxon possesses the mt haplogroup *aroa3*, which is the most differentiated within the entire *P. aroaniensis* species complex, and the

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Phylogenetic Structure Revealed through Combining DNA Barcodes with Multi-Gene Data for *Agrodiaetus* Blue Butterflies (Lepidoptera, Lycaenidae)

by Vladimir A. Lukhtanov^{1,*}, Nazar A. Shapoval^{1,*}, Alexander V. Dantchenko¹ and Wolfgang Eckweiler²

¹ Department of Karyosystematics, Zoological Institute, Russian Academy of Sciences, Universitetskaya Nab. 1, 199034 Saint-Petersburg, Russia

² Gronauer Street 40, D-60385 Frankfurt, Germany

* Authors to whom correspondence should be addressed.

Insects **2023**, *14*(9), 769; <https://doi.org/10.3390/insects14090769>

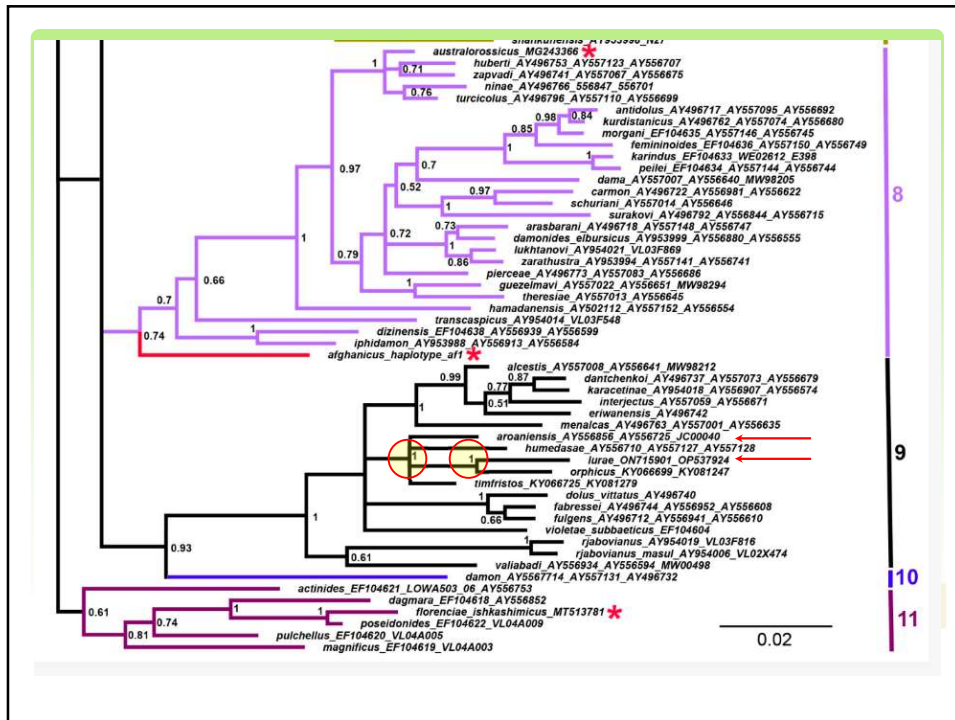
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(This article belongs to the Special Issue Systematics, Ecology and Evolution of Lepidoptera)

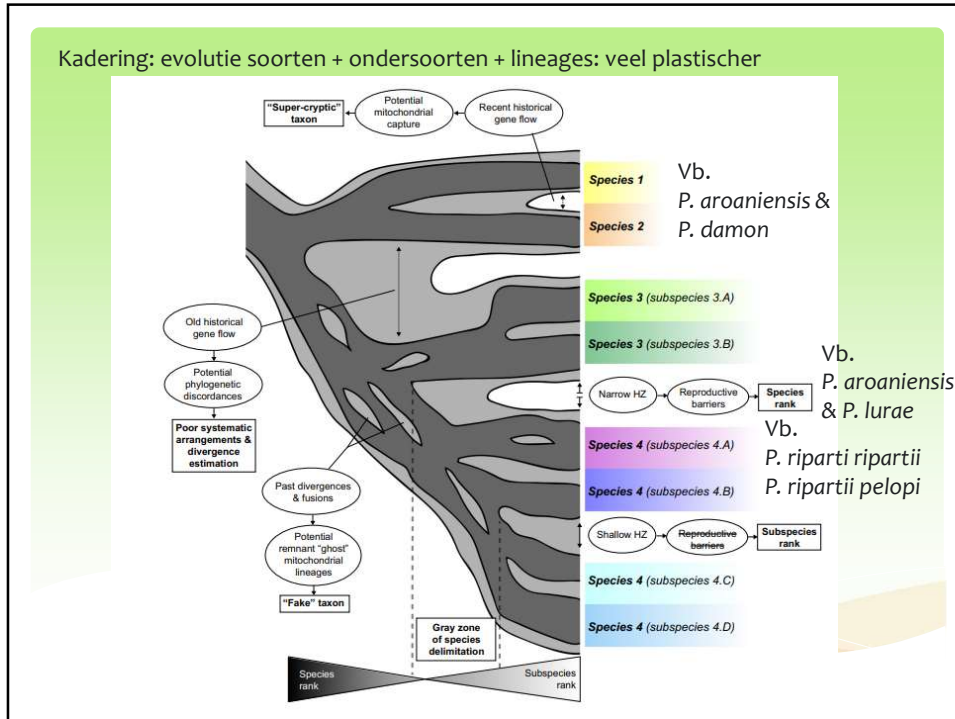
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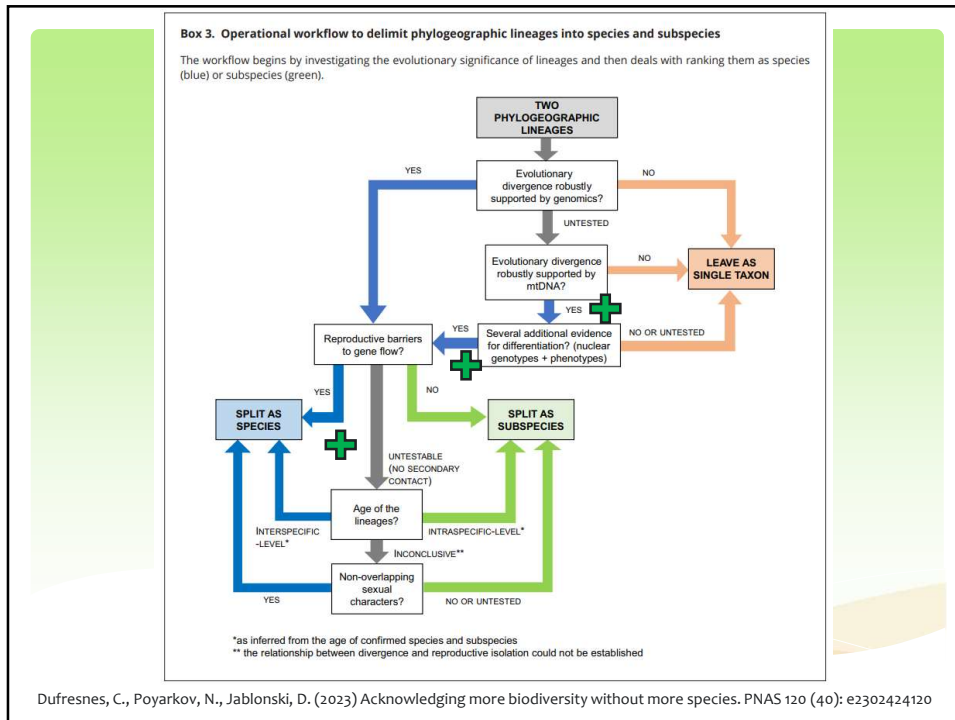
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P. lurae opgenomen in NCBI world database of taxa

NCBI Taxonomy Browser

Search for *Polyommatus lurae*

Display 3 levels using filter: none

Polyommatus lurae

Taxonomy ID: 2951542 (for references in articles please use NCBI:taxid:2951542)

current name: *Polyommatus lurae* Parmentier, Vila & Lukhtanov, 2022 in [Parmentier, I. et al. (2022)]
includes: *Polyommatus* sp. 1 I.P.-2022a

NCBI BLAST name: butterflies

Rank: species

Genetic code: Translation table 1 (Standard)

Mitochondrial genetic code: Translation table 4 (Invertebrate Mitochondrial)

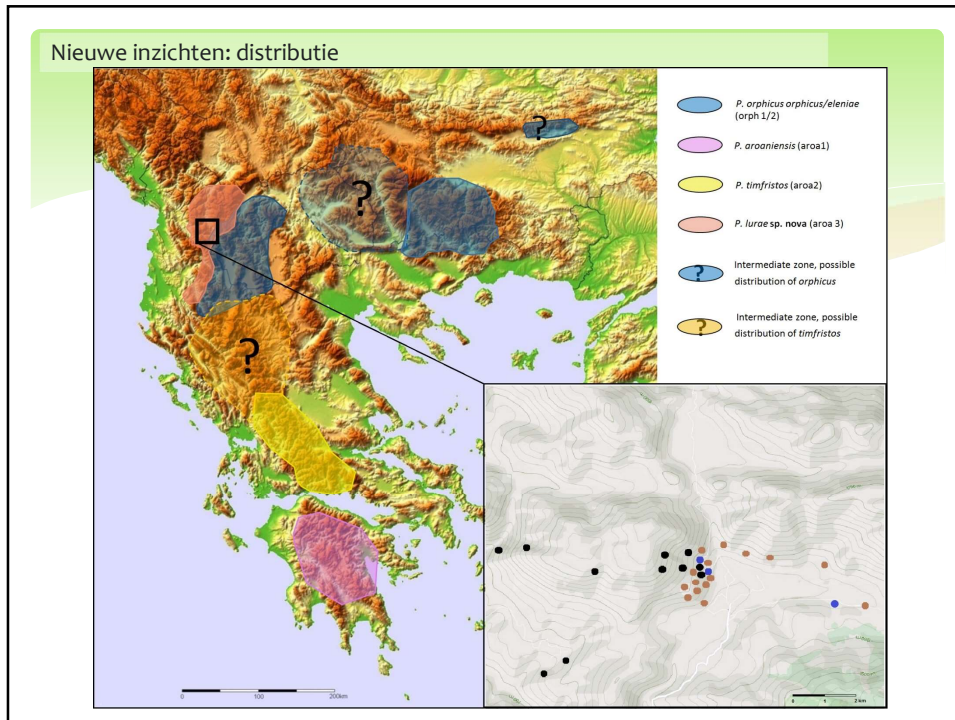
Lineage (full):
cellular organisms: Eukaryota: Opisthokonta: Metazoa: Eumetazoa: Bilateria: Protostomia: Ecdysozoa: Panarthropoda: Arthropoda: Mandibulata: Pancrustacea: Hexapoda: Insecta: Diptera: Pterygota: Neoptera: Endopterygota: Amphiesmenoptera: Lepidoptera: Glossata: Neolepidoptera: Heteroneura: Ditrysia: Obtectomera: Papilionoidea: Lycaenidae: *Polyommatus*: *Polyommatus*

Comments and References:
Parmentier, I. et al. (2022), doi: 10.3897/CompCytogen.v16i4.90558, PubMed: abstract
Parmentier I., Vila R., Lukhtanov V. 2022. Integrative analysis reveals cryptic speciation linked to habitat differentiation within Albanian populations of the anomalous blues (Lepidoptera, Lycaenidae, *Polyommatus* Latreille, 1804). *Comp. Cytogenet.* 16(4):211-212.



Information from sequence entries
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Nieuwe inzichten: distributie



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Thanks to...

Compagnons de route

- Delphine, Thomas, Xhulia,...



Wetenschappelijk onderzoeksteam

- Prof. Anila Papatisto (advies, UTirana, vergunningen)
- Prof. Roger Vila (barcoding, funding & review publicatie)
- Dr. Joan-Carles Hinojosa (fylogenetische analyse)
- Prof. Vladimir Lukhtanov (Karyotyping, funding & review publicatie)
- Anonieme reviewers
- Studenten Universiteit Tiranë - UGent

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- <https://compcytogen.pensoft.net/article/90558/list/11/>
- <https://www.mdpi.com/2075-4450/14/9/769>
- <https://www.pnas.org/doi/10.1073/pnas.2302424120>

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Thank you!

Questions?



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