Character-based DNA barcoding allows for integration of geography, ecology and morphology

Discovery of a cryptic species complex in dragonflies using CAOS

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ITZ, Ecology & Evolution
DNA barcoding

- 2 million described species
- Estimated number of species: 10-20 million or higher
- Estimated extinction rate: 0.25% per year

→ DNA Barcoding: perfect tool for fast identification of known species

Discovery of new species – Some problems

- Genetic distances variable between species groups
- No general threshold possible to diagnose new species
Distance-based DNA barcoding (COI)

Genetic distance between the two species:

1.8%

Genetic distances between species within this genus:

1.8 – 13%

Regarding the 3% rule

→ No separate species
Character-based barcoding

Definition of diagnostic characters of CO1 using CAOS (Character Attribute Organization System) (Sakar et al. 2002)

Advantage:

• Character matrix could be complemented with additional markers (for Odonates ND1 is highly suitable)
  and
• with characters of morphology, ecology, geography or others
CAOS barcoding

Character-based barcoding

<table>
<thead>
<tr>
<th>DNA</th>
<th>COI</th>
<th>ND1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>101</td>
<td>132</td>
</tr>
<tr>
<td>A. imperator</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td>A. parthenope</td>
<td>C</td>
<td>G</td>
</tr>
</tbody>
</table>

**Morphology**

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. imperator</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>A. parthenope</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Geography</th>
<th>Ecology</th>
</tr>
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<tbody>
<tr>
<td>A. imperator</td>
<td>Africa, Europe, Asia</td>
</tr>
<tr>
<td>A. parthenope</td>
<td>North Africa, Southern Europe, Asia</td>
</tr>
</tbody>
</table>

Two different species: supported by unambiguous characters of different disciplines
Population genetic analyses in *Trithemis stictica*

- widespread species in southern Africa
- permanent water pond with vegetation
Case study – Population genetics

Maximum Parsimony tree of ND1 sequences of 108 analysed "T. stictica" individuals

- Three genetic distinct groups
- High genetic distances
- No shared haplotypes
- Complete genetic isolation ($F_{st}$-values $\geq 0.89$)

Damm et al. (2010) Molecular Ecology
Are the three genetic clades already separate species?
Case study – Phylogenetic analyses

Bayesian analyses based on 16S, ND1, CO1 and ITS sequences

T. kirbyi

T. stictica (C1)

T. nuptialis

T. grouti

T. annulata

T. furva

T. spec. nov (C3)

T. spec. nov (C2)

ND1: 2.2%
CO1: 3.3%
16S: 1.2%
ITS: 1.0%

ND1: 5.0%
CO1: 5.7%
16S: 1.1%
ITS: 1.0%

• Genetic distances at the species level

• Confirmed by four different sequence markers

Damm et al. (2010) Molecular Ecology
## Case study – Character-based barcoding

<table>
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<tr>
<th>Pairwise comparison</th>
<th>ND1</th>
<th>COI</th>
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<tbody>
<tr>
<td>(T. \text{stictica} ,(C1) / ,T. \text{spec. nov.} ,(C2))</td>
<td>26</td>
<td>43</td>
</tr>
<tr>
<td>(T. \text{stictica} ,(C1) / ,T. \text{spec. nov.} ,(C3))</td>
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<td>43</td>
</tr>
<tr>
<td>(T. \text{stictica} ,(C1) / ,T. \text{nuptialis})</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>(T. \text{stictica} ,(C1) / ,T. \text{grouti})</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>(T. \text{spec. nov.} ,(C2)/ ,T. \text{spec. nov.} ,(C3))</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>(T. \text{spec. nov.} ,(C2) / ,T. \text{nuptialis})</td>
<td>32</td>
<td>51</td>
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<tr>
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### Pure diagnostic characters

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<tr>
<td>(T. \text{spec. nov.} ,(C2) / ,T. \text{nuptialis})</td>
<td>A: T A G T T T T</td>
<td>B: G T A A T T T</td>
</tr>
<tr>
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Damm et al. (2010) Molecular Ecology
Case study – Character-based barcoding

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High number of pure diagnostic characters in a pairwise comparison

Combination of species specific characters

<table>
<thead>
<tr>
<th>COI</th>
<th>Nucleotide positions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 144 162 180 279 288 294 297 330 333 360 393 396 454 459</td>
</tr>
<tr>
<td>Species</td>
<td></td>
</tr>
<tr>
<td><em>T. stictica</em> (C1)</td>
<td>C    C    A    C    T    A    A    T    T    G    T    A    A    A    T</td>
</tr>
<tr>
<td><em>T. spec. nov.</em> (C2)</td>
<td>C    G    A    A    A    A    T    T    T    T    C    A    A    C    T</td>
</tr>
<tr>
<td><em>T. spec. nov.</em> (C3)</td>
<td>A    G    A    A    G    G    C    T    T    G    T    G    A    C    T</td>
</tr>
<tr>
<td><em>T. grouti</em></td>
<td>A    G    G    C    T    A    A    T    T    G    T    A    A    T    C</td>
</tr>
<tr>
<td><em>T. nuptialis</em></td>
<td>C    G    G    C    T    A    A    C    C    G    T    A    A    A    T</td>
</tr>
<tr>
<td><em>T. annulata</em></td>
<td>A    T    T    C    A    A    A    T    T    A    T    A    C    A    T</td>
</tr>
<tr>
<td><em>T. furva</em></td>
<td>A    A    A    T    T    A    A    A    T    T    A    T    T    T    T</td>
</tr>
</tbody>
</table>

Damm et al. (2010) Molecular Ecology

15 selected nucleotide positions of CO1 to distinguish between seven different *Trithemis* species
Case study – Morphology

*Trithemis stictica*  
*T. spec. nov.*  
*T. spec. nov.*
Trithemis stictica

Case study – Morphology

- Colouration of the eyes

Damm & Hadrys (2009)
International Journal of Odonatology
Case study – Morphology

- Colouration of the eyes
- Colour of the wing basis

Trithemis stictica

T. spec. nov.

T. spec. nov.

Damm & Hadrys (2009)
International Journal of Odonatology
Case study – Morphology

*Significant size differences*

**Trithemis stictica**  
**T. spec. nov.**  
**T. spec. nov.**

Damm & Hadrys (2009)  
International Journal of Odonatology
Case study – Morphology

Males secondary genitalia

→ Reproductive isolation

distal segment

T. spec. nov.  T. spec. nov.  T. stictica

Damm & Hadrys (2009)
International Journal of Odonatology
Case study – Geography

Caprivi region

- *Trithemis stictica*
- *T*. spec. nov.
- *T*. spec. nov.
Case study – Ecology

*Trithemis stictica*

- shaded habitat
- highlands and natural sources in mountain regions

Damm et al. (2010) Molecular Ecology
Case study – Ecology

- *Trithemis stictica*

  - shaded habitat
  - highlands and natural sources in mountain regions

- *T. spec. nov*

  - rivers with gallery forest
  - fast running waters

Damm et al. (2010) Molecular Ecology
**Trithemis stictica**

- shaded habitat
- highlands and natural sources in mountain regions

**T. spec. nov**

- rivers with gallery forest
- fast running waters

**T. spec. nov**

- open habitat
- swamp-like habitat

Damm et al. (2010) Molecular Ecology
**Integrative approach for species discovery**

<table>
<thead>
<tr>
<th>DNA</th>
<th>Morphology</th>
<th>Reproductive Isolation</th>
<th>Ecology</th>
<th>Geography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seq. div. ND1 CO1</td>
<td>diagnostics ND1 CO1</td>
<td>Size parameters Hw Bs Hw AbdL S4</td>
<td>Cornuti shape differences ND1 COI</td>
<td></td>
</tr>
<tr>
<td><strong>T. stictica / Clade 2</strong></td>
<td>9.0 7.9</td>
<td>26 43</td>
<td>* ** - -</td>
<td>significant 0.960 0.984</td>
</tr>
<tr>
<td><strong>T. stictica / Clade 3</strong></td>
<td>8.5 8.3</td>
<td>27 43</td>
<td>*** *** *** ** *</td>
<td>significant 0.944 0.966</td>
</tr>
<tr>
<td><strong>Clade 2 / Clade 3</strong></td>
<td>5.0 5.7</td>
<td>13 28</td>
<td>- - ** **</td>
<td>weak 0.906 0.921</td>
</tr>
</tbody>
</table>

Distance-based DNA barcoding → Two new species

Confirmed by

- High number of diagnostic characters
- Morphology
- Reproductive Isolation (directly and indirectly)
- Ecology
- Geography

Damm et al. (2010) Molecular Ecology
Conclusions

Character-based barcoding allows

• Identification of known species

• Reliable discovery of new species

• Comprehensive database by integrating characters of multiple disciplines

• Many important information for conserving biodiversity

→ Discovery of the first cryptic dragonfly complex in Odonates
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• Jens Kipping
• Viola Clausnitzer

• BMBF BIOTA South
Thank you for your attention!