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Taxonomic Assessment of Andrena rosae and A stragulata by DNA-Sequencing (Hymenoptera: Apoidea: Andrenidae)

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Two taxa of the genus *Andrena* Fabricius 1775 (subgenus *Hoplandrena* Pérez 1890): *A rosae* Panzer 1801 and *A stragulata* Illiger 1806, are considered species by some authors; others consider *A stragulata* as the early spring generation of *A rosae*. In this paper the taxonomical status of both taxa is studied by comparing nuclear and mitochondrial DNA-sequences of spring and summer specimens from the Biesbosch National Park in the Netherlands. Despite the differences in morphology, eidonomy and nesting sites, no consistent differences could be found in the DNA-sequences of both supposed species. Therefore, *Andrena rosae* is considered to be a bivoltine species: with a spring and a summer generation. *Andrena stragulata* is considered a junior synonym of *A rosae*. This view is also supported by the distributional data of spring and summer specimens in the Netherlands.

Key words: *Andrena eximi*a Smith 1847 – *A rosae* Panzer 1801 – *A stragulata* Illiger 1806 – synonyms – spring generation – summer generation – Netherlands – DNA – COI

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Zwei Taxa der Gattung *Andrena* Fabricius 1775 (Untergattung *Hoplandrena* Pérez 1890): *A rosae* Panzer 1801 und *A stragulata* Illiger 1806, werden von manchen Verfassern als gute Arten angesehen; andere betrachten *A stragulata* als die Frühlingsgeneration von *A rosae*. In dieser Arbeit wird der taxonomische Status beider Formen durch den Vergleich von DNA-Sequenzen von Frühlings- und Sommerexemplaren aus dem Biesbosch im Südwesten der Niederlande untersucht. Trotz bestehender Unterschiede in Morphologie, Biologie und Ökologie zwischen den postulierten Arten konnten keine konstanten DNA-Differenzen gefunden werden. Deshalb wird *Andrena rosae* hier als eine bivoltine Art betrachtet: mit einer Frühlings- und einer Sommergeneration. *Andrena stragulata* wird somit ein jüngeres Synonym von *A rosae*. Diese Schlußfolgerung wird unterstützt durch Angaben über die Verbreitung von Fühlings- und Sommerexemplaren in den Niederlanden.

Stichwörter: *Andrena eximi*a Smith 1847 – *A rosae* Panzer 1801 – *A stragulata* Illiger 1806 – Synonyme – Frühlings-Generation – Sommer-Generation – Niederlande – DNA – COI

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1 Introduction

The solitary mining bees *Andrena stragulata* Illiger 1806 (= *A eximia* Smith 1847) and *A rosae* Panzer 1801 are West-Palaearctic taxa, occurring in Central and South-Europe. *A stragulata* can be found in early spring, while the flight period of *A rosae* is in summer. Some authors regard both taxa as two distinct, univoltine (= one generation per year) species [WESTRICH 1989, SCHMID-EGGER & SCHEUCHL 1997, GUSENLEITNER & SCHWARZ 2002]. Despite small differences in the male morphology [SCHMID-EGGER & SCHEUCHL 1997], other authors disagree and consider *A stragulata* and *A rosae* to be a single species with two generations per year [bivoltine: VAN DER VECHT 1928, PEETERS et al 1999].

In Dutch literature only the name *Andrena rosae* has been used, both for spring and summer specimens. In the Netherlands it is a rare species (**Fig 1**). It used to be more widely distributed before 1980, but after a strong decline it is now regarded as severely threatened in the national Red List of threatened species [PEETERS et al 1999, PEETERS & REEMER 2003]. In recent years, the Dutch population is concentrated in a limited area in the southwestern part of the Netherlands (**Fig 1**). In this area, both spring (*A stragulata*) and summer generations (*A rosae*) have been found in large numbers. Therefore this population provided a good opportunity to investigate the taxonomic status of both taxa. Additional information on the ecology of the population, including descriptions of nesting sites and notes on pollen collection and behaviour, can be found in VAN DER MEER et al [2006].

Fig 1: Distribution of *Andrena rosae* Panzer 1801 in the Netherlands. Squares: records before 1980. Dots: records between 1980 and 2006. Source: database of European Invertebrate Survey – the Netherlands.

This study must be done in two parts. In the first part, a limited number of specimens to be studied to determine which of four genetic markers is most informative. In the ond part, only COI-sequences have to be analyzed of a larger number of specimens. It ypothesized that if the total number of 39 specimens of spring and summer generations eed represent different species, then cladistic analysis should likewise group these spe- s in separate clades.

Materials and methods

Research area and collection of specimens

Male and female specimens of both spring and summer generations of *Andrena rosae* were lected from four localities in the National Park "De Biesbosch", an area in the southwest of the herlands. **Tab 1** lists the names and mutual distances of these localities. Specimens of *Andrena antonica* Pérez 1902 were used as outgroup. **Tab 2** lists the collection data of both ingroup and group specimens.

	DE	LD	TP	ZP
DE	–	–	–	–
LD	0.1	–	–	–
TP	1.7	1.8	–	–
ZP	7.7	7.7	7.3	–

Tab 1: Distances in kilometers between research localities in the Biesbosch (Netherlands). Values in parentheses indicate the coordinates in the 'Amersfoort'-grid, a national geographical grid-system in the Netherlands. DE= De Elzen (106,3–419,2); LD = Louisapolderse Dijk (106,3–419,3); TP = Zuiddijk bij Tongplaat (107,8–418,3); ZP = Zuilespolder (112,2–424,2)

After capturing by net the specimens were stored in 96 % ethanol and were identified using the s of Schmid-Egger & Scheuchl [1997]. All specimens are deposited in the collection of the Na- al Museum of Natural History (RMNH) in Leiden, the Netherlands. Specimen registration codes the DNA voucher specimens are included in **Tab 2**.

DNA extraction, PCR and sequencing

For DNA extraction of specimens collected in 2005 and 2006 (**Tab 2**) the abdomens were par- ly used; of the specimens collected in 2007, 2–3 legs were used. All vouchers were deposited at National Museum of Natural History Naturalis, Leiden, The Netherlands. Total genomic DNA was racted with a DNeasy® Tissue Kit (Qiagen) following the manufacturers protocol. To concentrate DNA, it was eluted in a final volume of 100 µl (buffer AE).

In the first part of the study six specimens of '*A rosae*' (three of each generation, all females) and r outgroup specimens of *A carantonica* Pérez 1902 were studied (**Tab 2**). For these six specimens, gments of COI, COII, CytB and EF-1α were sequenced. For the 33 additional specimens (2006 2007) only COI was sequenced. Primer information and references are listed in **Tab 3**. For all rkers the PCR conditions were 1.5 mM $MgCl_2$, 0.2 mM DNTPs, 0.4 µM of each primer and 5 U Taq DNA polymerase (Qiagen) per reaction. The only exception being the use of 2.0 mM $MgCl_2$ EF-1α. PCR amplifications were always carried out in a volume of 25 µl.

This study must be done in two parts. In the first part, a limited number of specimens has to be studied to determine which of four genetic markers is most informative. In the second part, only COI-sequences have to be analyzed of a larger number of specimens. It is hypothesized that if the total number of 39 specimens of spring and summer generations indeed represent different species, then cladistic analysis should likewise group these species in separate clades.

2 Materials and methods

2.1 Research area and collection of specimens

Male and female specimens of both spring and summer generations of *Andrena rosae* were collected from four localities in the National Park "De Biesbosch", an area in the southwest of the Netherlands. **Tab 1** lists the names and mutual distances of these localities. Specimens of *Andrena carantonica* Pérez 1902 were used as outgroup. **Tab 2** lists the collection data of both ingroup and outgroup specimens.

	DE	LD	TP	ZP
DE	–	–	–	–
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TP	1.7	1.8	–	–
ZP	7.7	7.7	7.3	–

Tab 1: Distances in kilometers between research localities in the Biesbosch (Netherlands). Values in parentheses indicate the coordinates in the 'Amersfoort'-grid, a national geographical grid-system in the Netherlands. DE= De Elzen (106,3–419,2); LD = Louisapolderse Dijk (106,3–419,3); TP = Zuiddijk bij Tongplaat (107,8–418,3); ZP = Zuilespolder (112,2–424,2)

After capturing by net the specimens were stored in 96 % ethanol and were identified using the keys of Schmid-Egger & Scheuchl [1997]. All specimens are deposited in the collection of the National Museum of Natural History (RMNH) in Leiden, the Netherlands. Specimen registration codes for the DNA voucher specimens are included in **Tab 2**.

2.2 DNA extraction, PCR and sequencing

For DNA extraction of specimens collected in 2005 and 2006 (**Tab 2**) the abdomens were partially used; of the specimens collected in 2007, 2–3 legs were used. All vouchers were deposited at the National Museum of Natural History Naturalis, Leiden, The Netherlands. Total genomic DNA was extracted with a DNeasy® Tissue Kit (Qiagen) following the manufacturers protocol. To concentrate the DNA, it was eluted in a final volume of 100 µl (buffer AE).

In the first part of the study six specimens of '*A rosae*' (three of each generation, all females) and four outgroup specimens of *A carantonica* Pérez 1902 were studied (**Tab 2**). For these six specimens, fragments of COI, COII, CytB and EF-1α were sequenced. For the 33 additional specimens (2006 and 2007) only COI was sequenced. Primer information and references are listed in **Tab 3**. For all markers the PCR conditions were 1.5 mM $MgCl_2$, 0.2 mM DNTPs, 0.4 µM of each primer and 5 U of Taq DNA polymerase (Qiagen) per reaction. The only exception being the use of 2.0 mM $MgCl_2$ for EF-1α. PCR amplifications were always carried out in a volume of 25 µl.

Tab 2: Collection and registration data of specimens of *Andrena rosae* Panzer 1801 from the Biesbosch area which have been used for DNA-sequencing, including four specimens of *A. carantonica* Pérez 1902 which have been used as outgroup. Specimen code: the first two letters form acronyms of the localities (explanation in table 1). Leg (collector): FM = Frank van der Meer; JS = John T. Smit; MR = Menno Reemer; TP = Theo M.J. Peeters. Voucher specimens are deposited in the collection of the RMNH (Leiden). The column *Study* indicates in which part(s) of the study the specimen has been used.

Specimen code	RMNH registration code	GenBank accession numbers	Season	Sex	Study	Date	Leg
DE-01-Spr-F	RMNH.INS.100001	COI: EU374640 COI-II: EU374680 CytB: EU374690 EF: EU374700	spring	female	1, 2	2.iv.2005	FM
DE-02-Spr-F	RMNH.INS.100002	COI: EU374641 COI-II: EU374681 CytB: EU374691 EF: EU374701	spring	female	1, 2	2.iv.2005	FM
DE-03-Spr-F	RMNH.INS.100003	COI: EU374642 COI-II: EU374682 CytB: EU374692 EF: EU374702	spring	female	1, 2	2.iv.2005	MR
DE-16-Spr-F	RMNH.INS.100004	COI: EU374659	spring	female	2	2.iv.2007	FM & JS
DE-17-Spr-F	RMNH.INS.100005	COI: EU374660	spring	female	2	2.iv.2007	FM & JS
LD-06-Spr-M	RHNH.INS.100006	COI: EU374649	spring	male	2	2.iv.2007	FM & JS
LD-07-Spr-M	RMNH.INS.100007	COI: EU374650	spring	male	2	2.iv.2007	FM & JS
LD-08-Spr-M	RMNH.INS.100008	COI: EU374651	spring	male	2	2.iv.2007	FM & JS
LD-09-Spr-M	RMNH.INS.100009	COI: EU374652	spring	male	2	2.iv.2007	FM & JS
LD-10-Spr-M	RMNH.INS.100010	COI: EU374653	spring	male	2	2.iv.2007	FM & JS
LD-65-Sum-F	RMNH.INS.100011	COI: EU374665	summer	female	2	23.vii.2007	MR
LD-66-Sum-F	RMNH.INS.100012	COI: EU374666	summer	female	2	23.vii.2007	MR
LD-67-Sum-F	RMNH.INS.100013	COI: EU374667	summer	female	2	23.vii.2007	MR
LD-71-Sum-F	RMNH.INS.100014	COI: EU374671	summer	female	2	23.vii.2007	MR
LD-72-Sum-F	RMNH.INS.100015	COI: EU374672	summer	female	2	23.vii.2007	MR
TP-01-Spr-F	RMNH.INS.100016	COI: EU374644	spring	female	2	2.iv.2007	FM & JS
TP-02-Spr-F	RMNH.INS.100017	COI: EU374645	spring	female	2	2.iv.2007	FM & JS
TP-03-Spr-M	RMNH.INS.100018	COI: EU374646	spring	male	2	2.iv.2007	FM & JS
TP-04-Spr-M	RMNH.INS.100019	COI: EU374647	spring	male	2	2.iv.2007	FM & JS
TP-05-Spr-F	RMNH.INS.100020	COI: EU374648	spring	female	2	2.iv.2007	FM & JS
TP-64-Sum-M	RMNH.INS.100021	COI: EU374664	summer	male	2	23.vii.2007	MR
TP-70-Sum-F	RMNH.INS.100022	COI: EU374670	summer	female	2	23.vii.2007	MR

TP-73-Sum-F	RMNH.INS.100023	COI: EU374673	summer	female	2	23.vii.2007	MR
TP-76-Sum-F	RMNH.INS.100024	COI: EU374676	summer	female	2	23.vii.2007	MR
TP-78-Sum-F	RMNH.INS.100025	COI: EU374677	summer	female	2	23.vii.2007	MR
TP-79-Sum-F	RMNH.INS.100026	COI: EU374678	summer	female	2	23.vii.2007	MR
ZP-04-Sum-F	RMNH.INS.100027	COI: EU374661 COI-II: EU374683 CytB: EU374693 EF: EU374703	summer	female	1, 2	14.vii.2005	MR
ZP-05-Sum-F	RMNH.INS.100028	COI: EU374662 COI-II: EU374684 CytB: EU374694	summer	female	1, 2	14.vii.2005	MR
ZP-06-Sum-F	RMNH.INS.100029	COI: EU374663 COI-II: EU374685 CytB: EU374695 EF: EU 374704	summer	female	1, 2	14.vii.2005	MR
ZP-07-Spr-M	specimen lost	COI: EU374643	spring	male	2	21.iv.2006	FM
ZP-11-Spr-F	RMNH.INS.100030	COI: EU374654	spring	female	2	2.iv.2007	FM & JS
ZP-12-Spr-F	RMNH.INS.100031	COI: EU374655	spring	female	2	2.iv.2007	FM & JS
ZP-13-Spr-F	RMNH.INS.100032	COI: EU374656	spring	female	2	2.iv.2007	FM & JS
ZP-14-Spr-F	RMNH.INS.100033	COI: EU374657	spring	female	2	2.iv.2007	FM & JS
ZP-15-Spr-M	RMNH.INS.100034	COI: EU374658	spring	male	2	2.iv.2007	FM & JS
ZP-68-Sum-M	RMNH.INS.100035	COI: EU374668	summer	male	2	23.vii.2007	MR
ZP-69-Sum-F	RMNH.INS.100036	COI: EU374669	summer	female	2	23.vii.2007	MR
ZP-74-Sum-F	RMNH.INS.100037	COI: EU374674	summer	female	2	23.vii.2007	MR
ZP-75-Sum-F	RMNH.INS.100038	COI: EU374675	summer	female	2	23.vii.2007	MR
Outgroup (Andrena carantonica), 1 & 2 collected in Steenbergen (province of Noord-Brabant), 3 & 4 collected in Rijswijk (province of Zuid-Holland)							
ANDRCARA1	RMNH.INS.100040	COI-II: EU374686 CytB: EU374700 EF: EU 374705		male	1, 2	30.iv.2005	TP
ANDRCARA2	RMNH.INS.100041	COI-II: EU374687 CytB: EU374697 EF: EU 374706		female	1, 2	30.iv.2005	TP
ANDRCARA3	RMNH.INS.100042	COI-II: EU374688 CytB: EU374698 EF: EU 374707		male	1, 2	14.v.2005	FM
ANDRCARA4	RMNH.INS.100043	COI-II: EU374679 CytB: EU374689 EF: EU 374699		female	1, 2	14.v.2005	FM

The same thermocycle profile was used of 3 minutes at 94 °C followed by 40 cycles of 30 seconds at 94 °C, 1 minute at 50 °C and 1 minute at 72 °C and a final extension of 5 minutes at 72 °C for all amplifications. PCR products were directly purified with Nucleospin® Extract II columns from Macherey-Nagel and both strands were sequenced using the same primers (**Tab 3**). Sequencing was either done on an ABI 3730 automated sequencher (Applied Biosystems) by Macrogen Corp in Korea or at Leiden University on a Megabace™ 1000 DNA Analysis System (Amersham). Forward and reverse sequences were assembled and checked using Sequencher 4.2 (Gene Codes Corp.). Sequences were aligned manually using MacClade 4.08 [Maddison & Maddison 2003].

Tab 3: Primer information.

Target	Primer name	Sequence	Direction	Source
Cytochrome c oxidase subunit I	LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'	Forward	Folmer et al, 1994
Cytochrome c oxidase subunit I	HCO2198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	Reverse	Folmer et al, 1994
Cytochrome c oxidase subunit I–II	'Jack'	5'-AGATCACTTGAATGATCACAAAAT-3'	Forward	Larkin et al, 2006
Cytochrome c oxidase subunit I–II	'Barb'/ C2-N-3661	5'-CCACAAATTTCTGAACATTGACCA-3'	Reverse	Simon et al, 1994
Cytochrome b	CytB F	5'-CGWTTAATTCATATAAATGG-3'	Forward	Koulianos et al, 1999
Cytochrome b	CytB R	5'-TATCATTCWGGTTTAATATG-3'	Reverse	Koulianos et al, 1999
Elongation Factor 1α	EF-1α F	5'-GGACACAGAGATTTCATCAARAA-3'	Forward	Kawakita et al, 2003
Elongation Factor 1α	EF-1α R	5'-TTGCAAAGCTTCRTGRTGCATTT-3'	Reverse	Kawakita et al, 2003

2.3 Sequence analysis

The datasets were analysed with PAUP 4.0 beta 10 [Swofford 2003]. To make a comparison between sequence variation within supposed species of *Andrena* ('*A rosae*') and between accepted species of *Andrena*, each of the four datamatrices of the first part of the study was checked for the number of variable and parsimony-informative sites within '*A rosae*' and between '*A rosae*' and *A carantonica* Pérez 1902. To visually cluster the specimens based on sequence similarity, an exhaustive search (optimality criterion: parsimony) and a Neighbor-Joining analysis (optimality criterion: distance) were performed on each of the pilot-datasets. Because a univocal split between spring and summer specimens of '*A rosae*' was only observed for marker COI, it was necessary to check whether the observed grouping was not caused by sampling errror (due to the restricted number of specimens). Hence the COI- dataset was extended to 40 taxa (including two outgroup specimens). On this extended dataset a NJ bootstrap analysis (1000 replicates) was carried out and a strict consensus tree was calculated.

Apart from the NJ analysis, a Minimum Spanning Network analysis has been performed on the sequence data. This did not provide any additional information, so these results are not included in this paper.

3 Results

3.1 Part 1: selection of informative markers

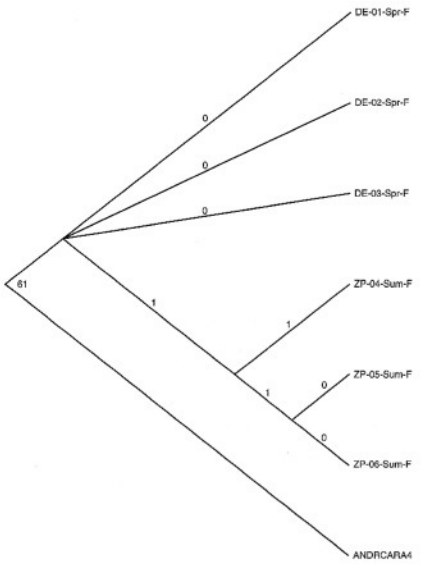
Sequences for all four markers were obtained for six specimens (except for an EF-1α sequence for specimen ZP-05-Sum-F). For marker COI and EF-1α some of the outgroup specimens occasionally failed, but each of the pilot datasets contained sequences of at least two *A. carantonica* specimens. For each dataset the results of an exhaustive search are shown in **Fig 2**. Only the exhaustive search on the CytB-dataset resulted in more than a single shortest tree; **Fig 2c** shows the 50 % majority rule consensus tree calculated of these five shortest trees. For both parsimony and distance analyses, the distance-measure was set to absolute.

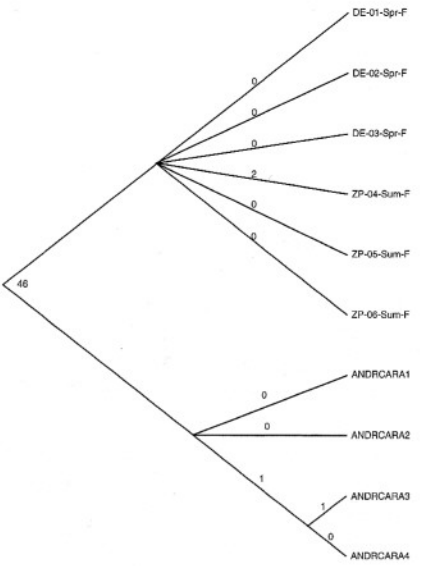
A summary of the number of variable and parsimony-informative sites for each dataset is given in **Tab 4**. This table also shows the effect of inclusion of *A carantonica* sequences on the total number of informative sites. The variation within COI-sequences of *A carantonica* was high compared to the variation of the other datasets analysed. Here a pseudogene might have been sequenced accidentially. Besides an unusually high sequence divergence for COI within *A carantonica* there were no other signs (i.e frame-shift mutations or stopcodons) indicating the presence of possible pseudogenes.

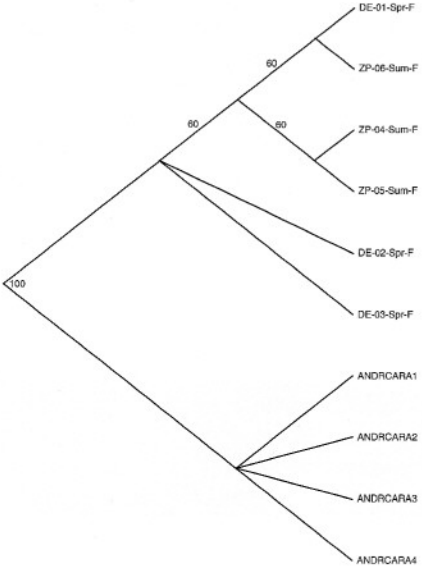
Tab 4 shows that the variation within '*A rosae*' is very limited. In fact, all sequences of '*A rosae*' in the COI-COII dataset are identical, as are those in the EF-1α dataset. The COI and CytB datasets each have two parsimony informative sites within '*A rosae*', but the CytB dataset results in a grouping of spring and summer specimens that is incompatible with our null-hypothesis (**Fig 2c**).

Tab 4: Character status (*Andrena carantonica* Pérez 1902 excluded / *A carantonica* included).

Dataset	Number of characters (primersites excluded)	Constant characters	Uninformative characters (Autapomorphies)	Parsimony-informative characters
COI	658	655 / 595	0 / 60	3 / 3
COI pilot	658	655 / 595	1 / 61	2 / 2
COI-COII pilot	689	687 / 639	2 / 3	0 / 47
CytB pilot	541	539 / 498	0 / 0	2 / 43
EF-1α pilot	782	782 / 776	0 / 2	0 / 4







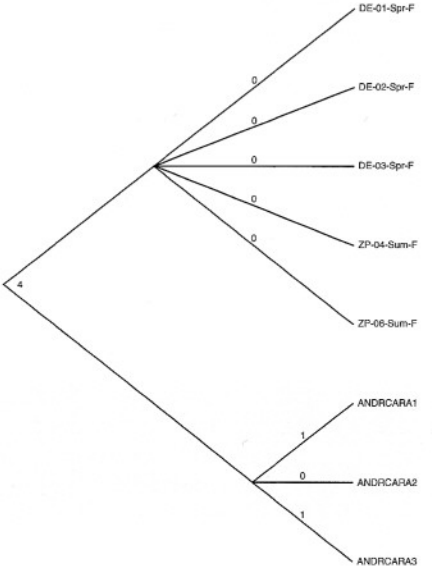


Fig 2: Cladistic analyses of sequences of four different genetic markers, as determined during the pilot-study for six specimens of *Andrena rosae* Panzer 1801 and one to four outgroup specimens of *A carantonica* Pérez 1902. **(a)** Cytochrome c oxidase subunit I (exhaustive search, shortest tree), **(b)** Cytochrome c oxidase subunit I–II (exhaustive search), **(c)** Cytochrome b (50% majority rule consensus, based on five shortest trees), **(d)** Elongation Factor 1α (exhaustive search shortest tree).

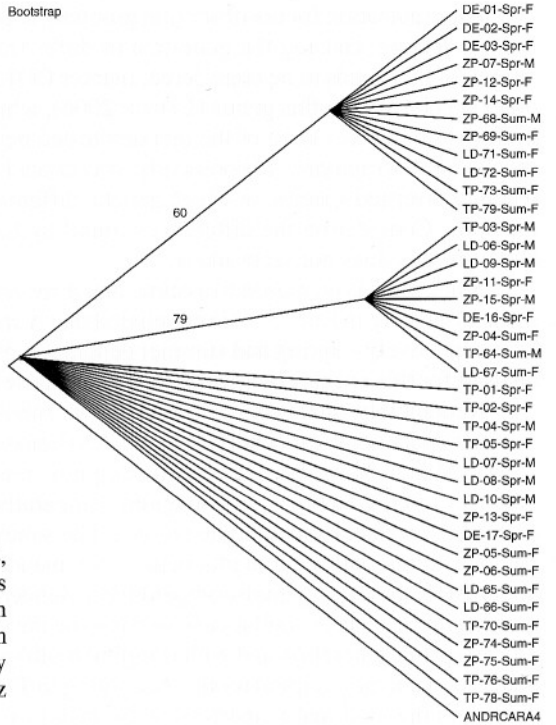


Fig 3: 50% majority rule consensus tree, based on COI-sequences of 39 specimens of *Andrena rosae* Panzer 1801 from both spring and summer generations, and from four different localities. The tree is rooted by a specimen of *Andrena carantonica* Pérez 1902 (ANDRCARA).

Although COI-COII sequences can discriminate between a large number of *Andrena*-species [LARKIN 2006], these sequences did not allow us to distinguish between spring and summer generations of the pilot specimens. In fact this pilot showed that except COI none of the tested markers could univocally discriminate between spring and summer generations. Hence only this potentially informative marker (COI) was used for the main study.

3.2 Part 2: analysis of COI in extended dataset

Although the grouping based on marker COI univocally supported our null-hypothesis (**Fig 2a**), the limited number of taxa made this dataset prone to sampling error. To check if these COI-clades had any statistical support, 33 specimens were added to the dataset. **Fig 3** shows the 50 % majority rule consensus tree of a neighbor joining bootstrap analysis (1000 replicates) of the extended COI-dataset. This cladogram shows that spring and summer specimens do not form separate clades. Moreover, even specimens from a single locality (presumably a single population) do not group. Each clade contains a mixture of specimens from different generations and localities.

4 Discussion

The here given null-hypothesis predicts that specimens of spring and summer generations would form different clades if they represented different species of *Andrena*. The results, however, neither show a grouping of specimens according to generation, nor according to location.

An explanation for not observing grouping according to generation could be incomplete lineage sorting; causing the gene-tree to differ from the species-tree. Although this is an argument that needs to be considered, marker COI-COII has shown to be discriminative for other species within this genus [Larkin 2006], while it clearly showed to be uninformative in this study. In fact none of the markers tested here enabled us to discriminate between *A rosae* and *A stragulata*. Theoretically, it is conceivable that we are dealing with two very recently diverged species, in which genetic differences are too small to detect with the used methods. Considering the differences found by Larkin [2006] between *Andrena* species, however, this does not seem likely.

Since there is no reason to assume that *A rosae* from the Netherlands is different from *A rosae* elsewhere in Europe, it is concluded that *A stragulata* and *A rosae* should be regarded as – respectively – spring and summer generation of the same species. The name that should be used for this species is *A rosae* Panzer, because this is the oldest available name.

Remarkably, there are considerable morphological differences between spring and summer specimens, especially in the males [Schmid-Egger & Scheuchl 1997]. The spring males (March–May) have a ventral occipital spine, mandibles longer and crossing each other for more than 60 %, hypopygium comparatively robust, eyes smaller, and more than half of second metasomal tergite reddish. The summer males (July–August) have no ventral occipital spine (but see remarks below), the mandibles shorter and crossing each other for about 10 %, the hypopygium comparatively slender, and less than half of the second metasomal tergite reddish. A similar case seems to be the species couple *Andrena spinigera* (Kirby 1802) (spring generation and with occipital tooth) and *A trimmerana* [Kirby 1802] (summer generation and no occipital tooth). According to Tadauchi & Hirashima [1984] the occipital spine and the modified mandibles of the males of the spring generation are typical for six Japanese species of the subgenus *Hoplandrena*, to which *A rosae* also belongs. They name the spring and summer generations "seasonal forms" and consider seasonal dimorphism normal for the subgenus in Japan. The males of the first generation in several species of this subgenus have a distinct occipital spine. It is the main character used by Schmid-Egger & Scheuchl [1997] to separate *A stragulata* and *A rosae*.

Although morphological analysis is beyond the scope of the present paper, some illustrations are included to demonstrate that the character states of the ventral occipital spine are not always clear-cut (**Fig 4**). In spring specimens this spine is always well-developed (**Fig 4a**), while in most summer specimens it is not (**Fig 4b**). However, in some summer specimens the state of this character is intermediate between spring specimens and usual summer specimens, as can be seen in **Fig 4c**. The variability of this character can be considered as additional support for our conclusion that there is only one species involved.

It is not clear how these morphological differences can develop among males of different generations within a single population. Another issue is whether these differences are functionally relevant, e.g in mating behaviour or ecology. Ecological research in the Netherlands has revealed ecological differences between spring and summer generations [Van der Meer et al 2006]. For instance, females collect pollen on *Salix* catkins in spring and on flowers of Apiaceae in summer. Another difference might be found in the nesting habits: while numerous nests were easy to locate in spring, no nests at all could be found at the same sites during summer, despite thorough searching and despite the presence of hundreds of specimens on near-by flowers. For the time being, however, one can only speculate about the causality of the seasonal morphological differences.

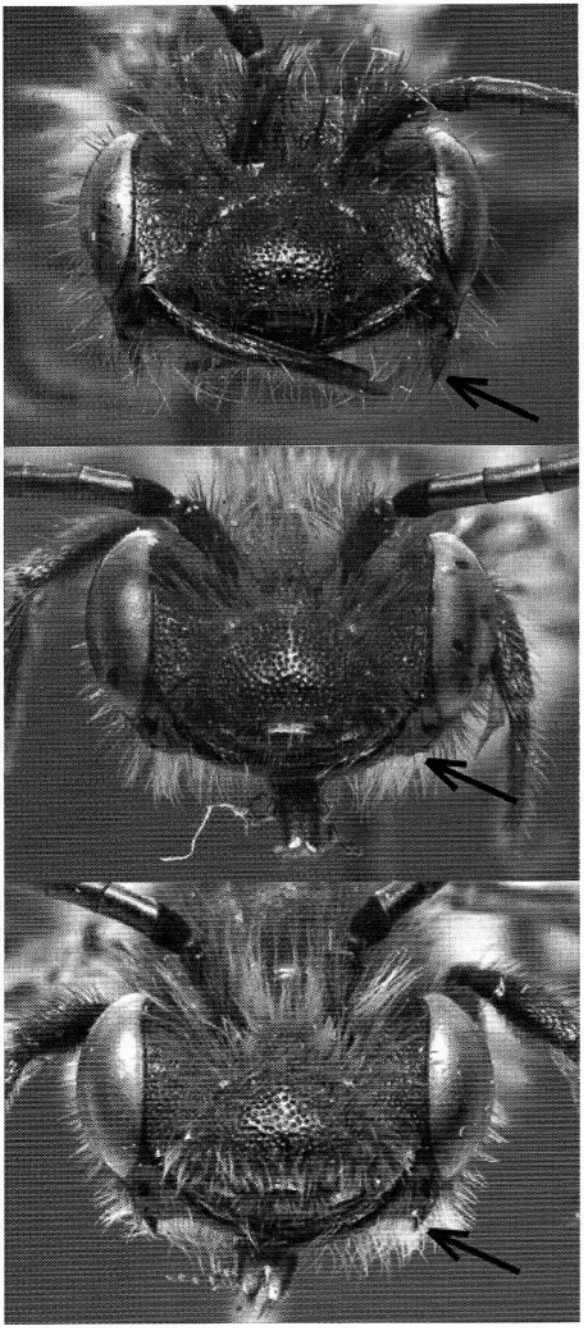


Fig 4: Head of male *Andrena rosae* Panzer 1801 in frontal view (all specimens from the Netherlands and deposited in collection of RMNH, Leiden). **(a)** Spring specimen with strongly developed ventral occipital spine (indicated by arrow): Maastricht, 22.iv.1954, leg. V. Lefeber. **(b)** Summer specimen without ventral occipital spine: Biesbosch, Hengstpolder, 29.vii.2005 (leg. M. Reemer). **(c)** Summer specimen with moderately developed ventral occipital spine, from same location and date as (b).

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