

On the female specimen *Nigma walckenaeri* (Roewer, 1951) misidentified as *Nigma puella* (Simon, 1870)

Erratum for

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Abstract

An erratum for HENRARD et al. (2022) is presented. The female dictynid specimen collected in the Jean Massart Botanical Garden (Brussels-Capital Region, Belgium), originally published as *Nigma puella* (Simon, 1870), has been morphologically re-examined and subjected to DNA-barcoding (COI) for species identification. The present investigation demonstrates that the specimen was misidentified, and is re-assigned to *Nigma walckenaeri* (Roewer, 1951).

Samenvatting

Een erratum voor HENRARD et al. (2022) wordt gepresenteerd. Het vrouwelijke exemplaar van het kaardertje verzameld in de botanische tuin Jean Massart (Brussels Hoofdstedelijk Gewest, België), oorspronkelijk gepubliceerd als *Nigma puella* (Simon, 1870), is morfologisch opnieuw onderzocht en onderworpen aan moleculaire barcoding (COI) voor genetische identificatie. De huidige studie toont aan dat het exemplaar verkeerd geïdentificeerd werd en eigenlijk *Nigma walckenaeri* (Roewer, 1951) betreft.

Résumé

Un erratum pour HENRARD et al. (2022) est présenté. Le spécimen femelle de dictynide collecté au Jardin botanique Jean Massart (Région de Bruxelles-Capitale, Belgique), publié à l'origine sous le nom de *Nigma puella* (Simon, 1870), a été réexaminé morphologiquement et soumis au barcoding moléculaire (COI) pour une identification génétique. La présente étude démontre que le spécimen a été mal identifié et qu'il s'agit en fait de *Nigma walckenaeri* (Roewer, 1951).

Introduction

The Jean Massart Botanical Garden, located in the Brussels-Capital Region of Belgium, displays an exceptional biodiversity. Previous studies have highlighted the impressive diversity of insects and other arthropods within this garden (PAULY 2019; DEKONINCK et al. 2019, 2023; GROOTAERT & DRUMONT 2022, 2023). This extensive biodiversity is also reflected in the arachnid fauna, with 239 species documented to date (HENRARD et al. 2022). Within the spider community found in the Jean Massart Botanical Garden, HENRARD et al. (2022) listed seven species for the family Dictynidae: *Brigittea latens* (Fabricius, 1775), *Dictyna arundinacea* (Linnaeus, 1758), *Dictyna pusilla* Thorell, 1856, *Dictyna uncinata* Thorell, 1856, *Lathys humilis* (Blackwall, 1855), *Nigma flavescens* (Walckenaer, 1830), and *Nigma puella* (Simon, 1870). However, *Nigma puella* is not yet included in the Belgian spider checklist (BOSMANS & VAN KEER 2017) and its identification in HENRARD et al. (2022) could potentially represent a new record for the Belgian arachnofauna that was previously overlooked.

The present study aims to validate this potential new record. Therefore, the specimen found in the Jean Massart Botanical Garden identified as *Nigma puella* has been re-examined. This study involves both morphological and DNA-based identification techniques.

Material and Methods

Material examined

Nigma walckenaeri (Roewer, 1951) (Araneae, Dictynidae):

BELGIUM • 1 ♀; Brussels, Oudergem, Jean Massart Botanical Garden; 50°48'50"N 4°26'17"E; 24.VIII.2016; C. Dekuijper, L. Dahan, H. Raemdonck & A. Drumont leg.; Beating vegetation; GenBank (COI): PP919328; RBINS_IG.33.177.

DNA-based species identification

DNA was extracted from two legs of the specimen using the QIAamp DNA Micro Kit (Qiagen, Venlo, the Netherlands) following the manufacturer's protocol (elution volume: 50 µl). The DNA extract was dried using the GenTegra technology after investigation and stored at room temperature at the Royal Belgian Institute of Natural Sciences (RBINS). The mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO-700ME (5'-TCAGGGTGACCAAAAAATCA-3') (FOLMER et al. 1994; BRETON et al. 2006), targeting a 664 bp long fragment. PCR conditions and protocol were as described in BORK (2015). PCR product and negative control were checked on a 1.5% agarose gel, using a UV transilluminator and the MidoriGreen™ Direct method (NIPPON Genetics Europe, Dueren, Germany). Positive COI amplification was subsequently purified using the ExoSAP-IT™ protocol (following manufacturer's instructions) and sequenced in both directions by Macrogen Europe (the Netherlands). Paired bi-directional strands were then trimmed and checked for stop codons using Geneious Prime® (Biomatters Ltd. Auckland, New Zealand). A consensus sequence was generated for the specimen, and subsequently compared against the Identification System of BOLD, with Species Level Barcode Records option (www.boldsystems.org).

Additionally, a Neighbour-Joining (NJ) tree (Tamura-Nei distance model, 1000 bootstrap replicates) was constructed to examine the clustering support of each species of the *Nigma* genus. To this end, the generated sequence was first aligned with all publicly available COI sequences of *Nigma* species downloaded from BOLD and GenBank (www.ncbi.nlm.nih.gov/genbank), using MUSCLE in Geneious Prime®. Then duplicates (i.e., identical sequences) were discarded per species to limit the size of the database. The final alignment comprised 18 unique COI sequences (see Appendix 1 for details). Among these sequences, the COI sequence of *Ajmonia gratiosa* (Simon, 1881) was included, as this species was recently retransferred from the genus *Nigma* to *Ajmonia* Caporiacco, 1934 (WUNDERLICH 2022). Additionally, COI sequences of *Dictyna uncinata* (FBARB313-11) and *Dictyna arundinacea* (ARBCM1230-14) from the Dictynidae family were included to root the NJ tree.

Examination and illustrations

The sample identified as "*Nigma puella*", a female specimen deposited at the Royal Belgian Institute of Natural Sciences (collection number RBINS_IG.33.177), was re-examined. Photographs of the specimen and genitalia immersed in ethanol 70% were taken with a DFC500 camera mounted on a Leica MZ16A and piloted with the Leica Application Suite software (LAS ver. 4.13). The epigyne was removed from the abdomen and digested using half a tablet of Total Care Enzima product (protein removal system originally for cleaning contact lenses and containing subtilisin A-0,4 mg per tablet; Abbott Medical Optics, Santa Ana, CA) in a few millilitres of distilled water overnight, then immersed back in 70% ethanol to be photographed. Some photos are available on the website "Les araignées de Belgique et de France" by Pierre Oger (<https://arachno.piwigo.com/>).

Results

DNA-based analyses

Using the Identification System of BOLD, a maximal match (100%) was obtained with a representative of the species *Nigma walckenaeri*, a voucher specimen collected in Germany (FBARB987-15; ASTRIN et al., 2016). The identification was further supported by the NJ tree construction, with a 1000 bootstrap support of the cluster including the generated sequence nested with other *Nigma walckenaeri* COI sequences downloaded from the online DNA repositories (Fig. 1). The generated COI sequences (1060 bp) is available on GenBank under the accession number PP919328.

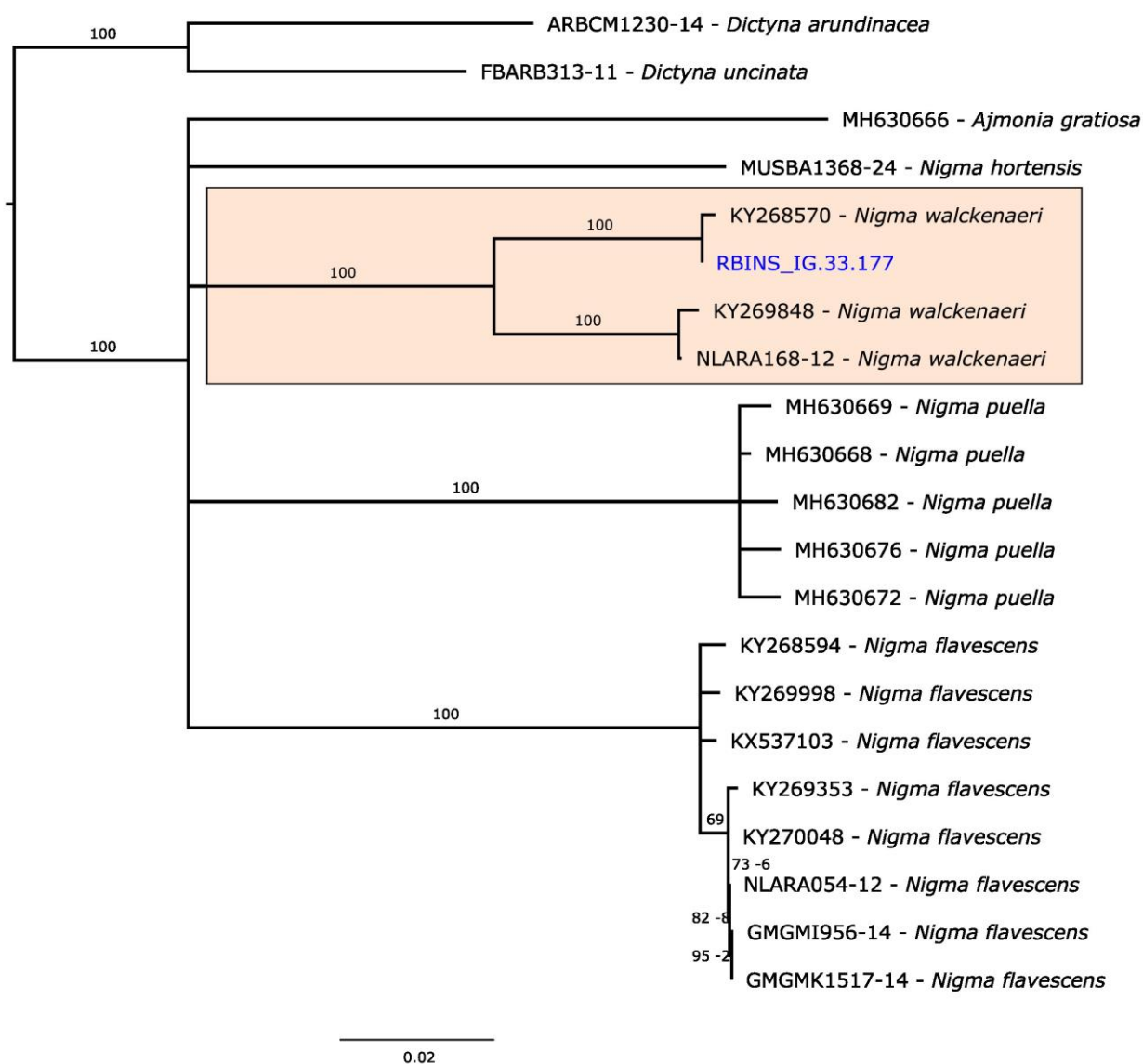


Figure 1: Neighbour-Joining tree including five species of the genus *Nigma* based on the cytochrome c oxidase subunit I (COI), with *N. flavescens*, and *N. walckenaeri* being the only species recorded in Belgium (Tamura-Nei distance model; 648 bp fragment; 1000 bootstrap replicates), and with *Dictyna uncinata* (FBARB313-11) and *Dictyna arundinacea* (ARBCM1230-14) as outgroups. The bootstrap values are shown at the branch points (sequence of query specimen highlighted in blue). Minimum branch support displayed is 65, other branches are collapsed.

Morphology

Morphological examination of the specimen preserved in alcohol reveals a very pale, yellowish-white coloration of the habitus (Fig. 2A-B). The observation of the genitalia required the dissection of the vulva (Fig. 2C-F). After clearing through the digestion process, it became more evident that the vulva corresponds to

Nigma walckenaeri, as seen in WIEHLE (1953: p. 80, fig. 171b) and LOKSA (1969: p. 39, fig. 30A). The vulva is provided with a pair of semi-translucent, tube-shaped structures and small, notched conical sclerites, widely separated from each other (see Figs 2C-F; vs. in *N. puella*, vulva with large conical sclerites, see BREITLING 2020: 342, fig. 7C and REHFELDT & CASSAR 2024: 22, fig. 7b).

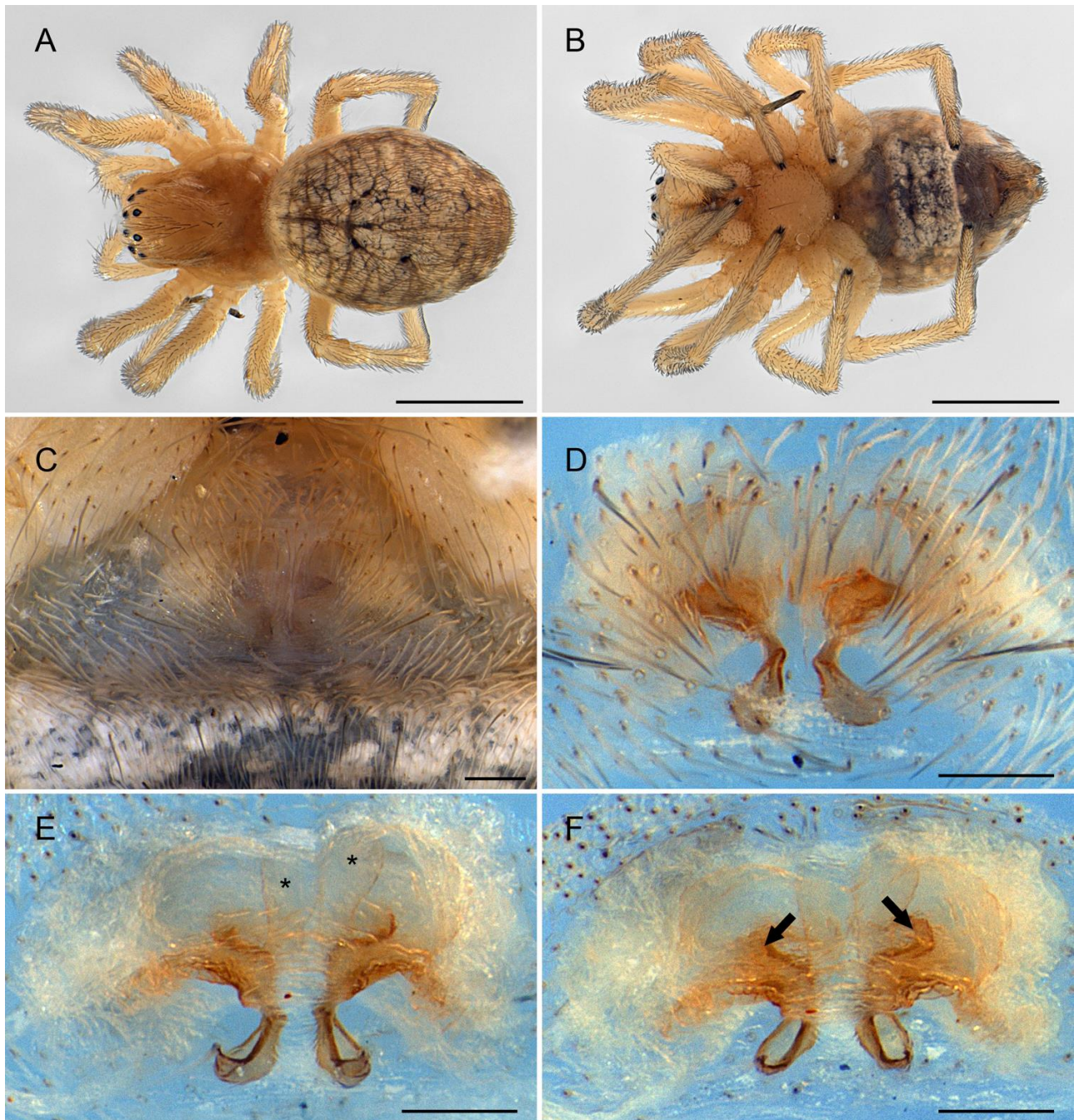


Figure 2: *Nigma walckenaeri* (Roewer, 1951), female specimen RBINS_IG.33.177) collected in the Jean Massart botanical garden (Brussels-Capital Region, Belgium). **A.** Habitus, dorsal view. **B.** Idem, ventral view. **C.** Epigastric region, ventral view. **D.** Vulva, ventral view. **E.** Idem, dorsal view. **F.** idem, slightly anterior view. The stars indicate the semi-translucent, tube-shaped structures and the arrows point to the notched conical sclerites. Scale bars: A-B = 1 mm; E-F = 0.1 mm.

Discussion

The specimen RBINS_IG.33.177 collected from the Jean Massart Botanical Garden exhibits a very pale coloration (Fig. 2A-B), which is often observed in *Nigma puella* and likely led to the initial misidentification. In contrast, living female specimens of *N. walckenaeri* typically display a distinctive flashy greenish coloration (Fig. 3A), with males also featuring a contrasting reddish prosoma (Fig. 3B). This vivid coloration strikingly

faded in the specimen due to its preservation in alcohol. The male is further identifiable by its palp with minute patella and tibial apophyses (Fig. 3D; see also ROBERTS 1995: p. 87), the embolus course with only a very faint subapical deviation (Fig. 3C) and the angular shape of conductor tip (Fig. 3D).

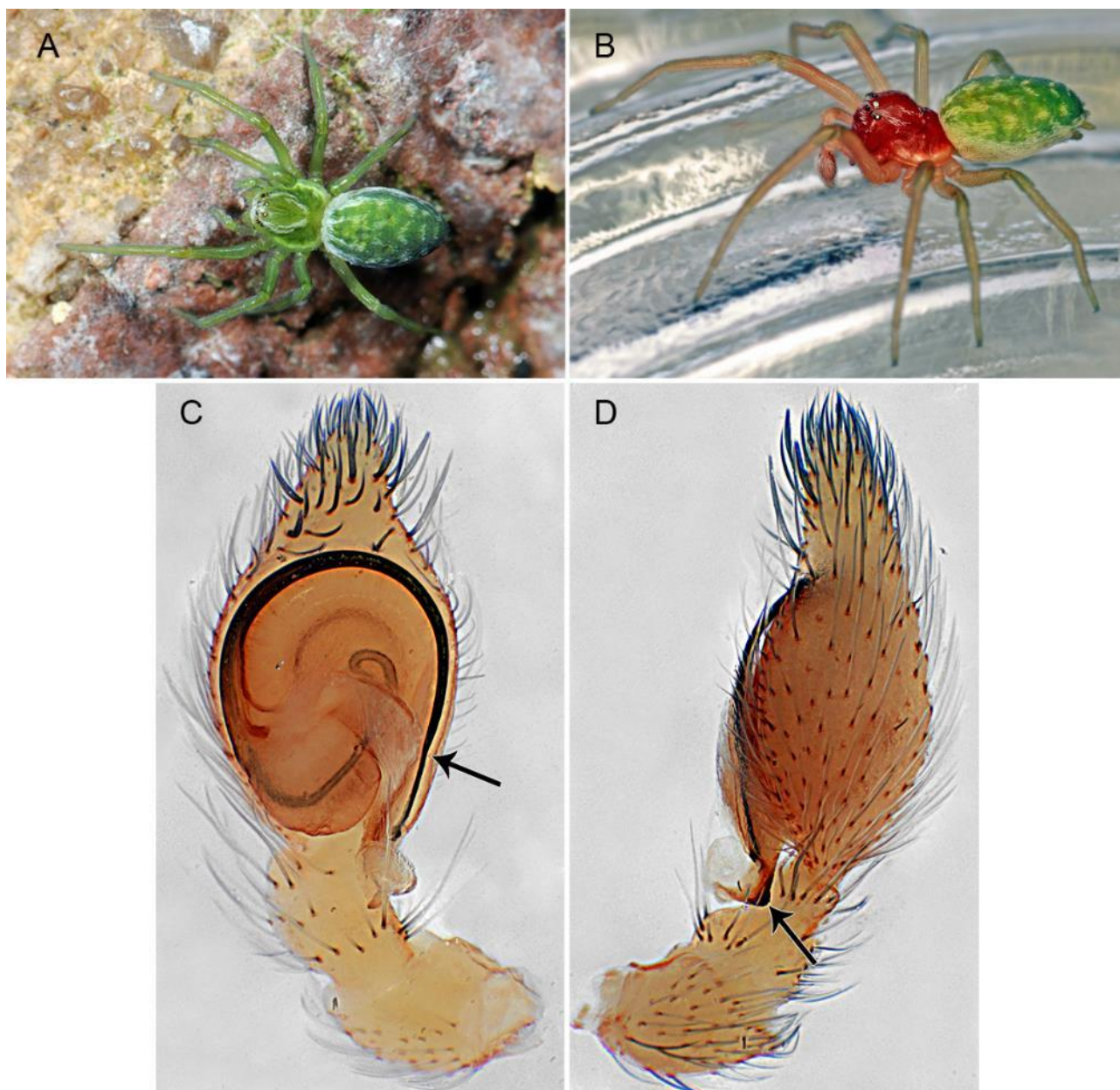


Figure 3: *Nigma walckenaeri* (Roewer, 1951). **A.** Living female from Waret-l'Évêque (Photo & coll. Pierre Oger). **B.** Living male from Houx (Yvoir) (Coll. AH_20100910_01). **C.** Idem, palp ventral view, the arrow pointing to the very faint subapical deviation of the embolus. **D.** Idem, retrolateral view, the arrow pointing to the angular shape of conductor.

The epigynes of *Nigma* species are challenging to interpret, and it is often necessary to rely on habitus information or to dissect the genitalia to examine the vulva's characteristics. Upon examining the vulva of the specimen, it became apparent that it did not align with the expected genital morphology of *N. puella*. Moreover, the habitus of *N. puella* typically features a contrasting dorsal red marking on the abdomen, which is absent in this specimen. These observations were validated by DNA-based identification techniques, resolving the ambiguity and conclusively identifying the specimen as *N. walckenaeri*. Although this remains the first mention for the botanical garden (the other recorded species being *N. flavescens*), this species is well-established in Brussels capital. It was even reported in 2020 in the vicinity of the Jean Massart Botanical Garden, in Oudergem, as documented on https://observations.be/species/8724/observations/?date_after=200-01-01&date_before=2024-07-09&country_division=19&search=Oudergem&user=&location=&sex=&month=&life_stage=&activity=&met

[hod=](#)). It is also worth noting that the adult activity periods of these two species differ (NENTWIG et al. 2024). *Nigma puella* is a spring/summer maturing species, with peak adult activity in May and June, while *N. walckenaeri* mostly matures in the autumn, with peak adult activity in September and October. The two species have only a slight overlap in their adult periods at the end of summer, which coincides with the collection date of the analysed specimen. In addition, both *Nigma* species built cribellate web on leaves of trees and shrubs in various environments (ROBERTS 1995, 1998; LE PERU 2007). However, *N. walckenaeri* seems to especially have a strong affinity for gardens and parks (ROBERTS 1995; Tony Russels-Smith, pers. comm.).

The Belgian checklist currently counts 15 Dictynid species (BOSMANS & VAN KEER 2017; NENTWIG et al. 2024; HENRARD et al. 2024), including two *Nigma* species: *N. flavescens* (Walckenaer, 1830) and *N. walckenaeri*. The species *Nigma puella* appears largely distributed in Europe (NENTWIG et al. 2024; (WORLD SPIDER CATALOG 2024), and it could only have been a matter of time before this species was reported from our country. Finally, its discovery in Belgium was documented based on a photograph taken by Louis Bronne the 01/06/2023 and uploaded on OBSERVATIONS.BE/WAARMENINGEN.BE (<https://observations.be/observation/275136815/>). The photo was then published in the issue 117 nov. dec. 2023 of the NATAGORA magazine. With new specimen collected in this locality by the first author, its presence in Belgium is now formally confirmed in the present journal issue, also validated by DNA-barcoding (HENRARD et al. 2024).

Acknowledgments

We are grateful to Pierre Oger for the use of his photographs of living female specimens of *Nigma walckenaeri*. We thank Tony Russell-Smith and Garben Logghe for proofreading this article. We also thank Pallieter De Smedt for his dedicated editorial contributions. The Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo – <https://www.bopco.be/>) is financed by the Belgian Science Policy Office (Belspo). The molecular data that support the findings of this study are openly available in GenBank at <https://www.ncbi.nlm.nih.gov/genbank/>, accession number: PP919328.

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Appendix 1. List of COI sequences.**Table 1:** List of COI sequences and species used in the Neighbour-Joining tree reconstruction, including voucher numbers, GenBank and/or BOLD accession numbers, and references. 'Shade' indicates the specimen sequenced during this study.

Species	Voucher ID	GenBank	BOLD	Reference
<i>Dictyna uncinata</i> Thorell, 1856	BC ZSM ARA 00313	KX536847	FBARB313-11	Astrin et al. 2016
<i>Dictyna arundinacea</i> (Linnaeus, 1758)	BIOUG14290-H07	KP653531	ARBCM1230-14	Blagoev et al. 2016
<i>Ajmonia gratiosa</i> (Simon, 1881)	UB-MD3340	MH630666	IBARA2422-18	Crespo et al. 2018
<i>Nigma hortensis</i> (Simon, 1870)	MNCN-ADN-200018	/	MUSBA1368-24	Unpublished
<i>Nigma walckenaeri</i> (Roewer, 1951)	ZFMK-TIS-20911	KY268570	GBBSP1028-15	Astrin et al. 2016
	ZFMK-TIS-7091	KY269848	GBBSP881-15	Astrin et al. 2016
	RMNH.ARA.12594	/	NLARA168-12	Unpublished
	RBINS_IG.33.177	PP919328	/	This study
<i>Nigma puella</i> (Simon, 1870)	UB-MD1916	MH630669	IBARA938-18	Crespo et al. 2018
	UB-MD1928	MH630668	IBARA951-18	Crespo et al. 2018
	UB-MD3341	MH630682	IBARA2423-18	Crespo et al. 2018
	UB-MD399	MH630676	IBARA2638-18	Crespo et al. 2018
	UB-MD1929	MH630672	IBARA952-18	Crespo et al. 2018
<i>Nigma flavescens</i> (Walckenaer, 1830)	ZFMK-TIS-18513	KY268594	GBBSP216-15	Astrin et al. 2016
	ZFMK-TIS-2516845	KY269998	GBBSP1439-15	Astrin et al. 2016
	BC ZSM ARA 00304	KX537103	FBARB304-11	Astrin et al. 2016
	ZFMK-TIS-7169	KY269353	GBBSP936-15	Astrin et al. 2016
	ZFMK-TIS-20892	KY270048	GBBSP365-15	Astrin et al. 2016
	RMNH.ARA.12510	/	NLARA054-12	Unpublished
	BIOUG17117-A01	/	GMGMI956-14	Unpublished
	BIOUG17226-F06	/	GMGMK1517-14	Unpublished