

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/388485326>

First record of *Nigma puella* (Simon, 1870) in Belgium, validated by genetic identification (Araneae, Dictynidae)

Article · December 2024

CITATIONS

0

READS

154

7 authors, including:



[Arnaud Henrard](#)

Royal Museum for Central Africa

65 PUBLICATIONS 439 CITATIONS

[SEE PROFILE](#)



[Garben Logghe](#)

Ghent University

15 PUBLICATIONS 11 CITATIONS

[SEE PROFILE](#)



[Louis Bronne](#)

Natagora

21 PUBLICATIONS 87 CITATIONS

[SEE PROFILE](#)



[Smitz Nathalie](#)

Royal Museum for Central Africa

87 PUBLICATIONS 441 CITATIONS

[SEE PROFILE](#)

First record of *Nigma puella* (Simon, 1870) in Belgium, validated by genetic identification (Araneae, Dictynidae)

Arnaud HENRARD¹, Garben LOGGHE^{2,3}, Louis BRONNE⁴, Nathalie SMITZ¹, Maël VANDERBERKEN⁵, Alix ATTAQUE⁶, Koen VAN KEER⁷

¹ Royal Museum for Central Africa, Biology Department, Leuvensesteenweg 13, 3038 Tervuren, Belgium. (e-mail: arnaud.henrard@africamuseum.be)

² Terrestrial Ecology Unit, Ghent University, Karel Lodewijk Ledeganckstraat 35, 9000 Ghent, Belgium

³ Research Institute for Nature and Forest (INBO), Herman Teirlinckgebouw, Havenlaan 88 Bus 73, 1000 Brussels, Belgium

⁴ Natagora (NGO), Impasse des Muses, 1, 5000 Namur, Belgium

⁵ UCLouvain, Faculty of Science, School of Biology, Place Croix du Sud 4-5 (box L7.07.05), 1348 LLN

⁶ Conservation Genetics Laboratory (GeCoLAB), University of Liège, Chemin de la vallée 4, 4000 Liège, Belgium

⁷ Boomgaardstraat 79, 2018 Antwerpen, Belgium

Abstract

The presence of the bleeding heart spider *Nigma puella* (Simon, 1870), a member of the Dictynidae family, had not been formally confirmed in Belgium until now. The species was recorded in Tihange in 2023 through a photograph posted on Observations.be / Waarmeningen.be, the Belgium participatory platform for monitoring biodiversity. Subsequently, in 2024, two males and a female were captured at the same site. Their identity is now confirmed through sequencing a fragment of their cytochrome c oxidase I (COI) gene and examining their morphological characteristics. A list of key characters is also presented for the three species of *Nigma* Lehtinen, 1967 found in Belgium. The list of Dictynid species occurring in Belgium is updated.

Samenvatting

De aanwezigheid van het bont kaardertje *Nigma puella* (Simon, 1870), een lid van de familie Dictynidae, was tot nu toe in België niet formeel bevestigd. De soort werd in 2023 in Tihange waargenomen via een foto die werd geplaatst op Observations.be / Waarmeningen.be, het Belgische participatieve platform voor het monitoren van biodiversiteit. Vervolgens werden in 2024 op dezelfde locatie twee mannetjes en een vrouwtje gevangen. Hun identiteit wordt nu bevestigd, op basis van de sequentiebepaling van een fragment van hun cytochroom c oxidase I (COI) gen en op het onderzoeken van hun morfologische kenmerken. Er wordt ook een lijst met sleutelkenmerken gepresenteerd voor de drie soorten van *Nigma* Lehtinen, 1967 die in België gevonden zijn. De lijst met in België voorkomende Dictynidae-soorten is bijgewerkt.

Résumé

La présence de l'araignée dictyne fille *Nigma puella* (Simon, 1870), apparentant à la famille des Dictynidae, n'avait jusqu'à présent pas été formellement confirmée en Belgique. L'espèce a été recensée à Tihange en 2023, via une photographie postée sur Observations.be / Waarmeningen.be, la plateforme participative belge pour la surveillance de la biodiversité. Par la suite, en 2024, deux mâles et une femelle ont été capturés sur le même site. Leur identité est maintenant confirmée par le séquençage d'un fragment de leur gène de la cytochrome c oxydase I (COI) et l'examen de leurs caractéristiques morphologiques. Une liste de caractères clés est également présentée pour les trois espèces de *Nigma* Lehtinen, 1967 rencontrées en Belgique. La liste des espèces de Dictynidae présentes en Belgique est mise à jour.

Introduction

Dictynidae is a family of small cribellate spiders, well distributed around the world and containing 461 species spread amongst 52 genera (WORLD SPIDER CATALOG 2024). Most species build irregular tangle webs among leaves of vegetation, on walls or on the ground. Since the spider checklist of BOSMANS & VAN KEER (2017), was published which included 17 species and nine genera of Dictynidae occurring in Belgium, some taxonomic

changes have occurred. The genus *Cicurina* Menge, 1871, containing the species *C. cicur* (Fabricius, 1793), was first moved to the Hahniidae by WHEELER et al. (2017) and then to its own family, Cicurinae, by GORNEAU et al. (2023). The genus *Mastigusa* Menge, 1854, comprising the species *M. arietina* (Thorell, 1871) and *M. macrophthalma* (Kulczyński, 1897) was transferred from the Dictynidae to the Hahniidae by WHEELER et al. (2017) and then to the Cybaeidae by CASTELLUCCI et al. (2023). In the meantime, the latter species was removed from the Belgian spider checklist (CASTELLUCCI et al. 2024; NENTWIG et al. 2024). Finally, the species *Argyroneta aquatica* (Clerck, 1757) was transferred from the Cybaeidae to the Dictynidae by WHEELER et al. (2017), although its placement is still debated (ONO & OGATA 2018).

Among Dictynidae occurring in Belgium, the genus *Nigma* Lehtinen, 1967 currently includes two species: *N. flavescens* (Walckenaer, 1830) and *N. walckenaeri* (Roewer, 1951). The species *Nigma puella* has only recently been observed in Belgium and documented for the first time, based on a photograph taken on 01/06/2023 near the Tihange Nuclear Power Station and published in OBSERVATIONS.BE / WAARMENINGEN.BE (<https://observations.be/observation/275136815/>). The photo was also published in issue 117 of the Natagora magazine (November 2023; <https://www.natagora.be/nos-publications>).

In June 2024, adult specimens were collected from the same locality, and subsequently identified by DNA-barcoding and morphological characterization. Using both identification methods, this paper formally confirms the presence of *N. puella* in Belgium. Therefore, the Belgian checklist of the family Dictynidae is updated and now includes 16 species distributed across eight genera (Table 1).

Table 1: Species list of Dictynidae found in Belgium. 'Shade' indicates the species sequenced during this study.

Genus	Species
<i>Argyroneta</i>	<i>aquatica</i> (Clerck, 1757)
<i>Altella</i>	<i>lucida</i> (Simon, 1874)
<i>Argenna</i>	<i>patula</i> (Simon, 1874)
	<i>subnigra</i> (O. Pickard-Cambridge, 1861)
<i>Brigittea</i>	<i>civica</i> (Lucas, 1848)
	<i>latens</i> (Fabricius, 1775)
<i>Dictyna</i>	<i>arundinacea</i> (Linnaeus, 1758)
	<i>major</i> Menge, 1869
	<i>pusilla</i> Thorell, 1856
	<i>uncinata</i> Thorell, 1856
<i>Emblyna</i>	<i>brevidens</i> (Kulczyński, 1897)
<i>Lathys</i>	<i>humilis</i> (Blackwall, 1855)
	<i>stigmatisata</i> (Menge, 1869)
<i>Nigma</i>	<i>flavescens</i> (Walckenaer, 1830)
	<i>puella</i> (Simon, 1870)
	<i>walckenaeri</i> (Roewer, 1951)

Material and Methods

Material studied

Nigma puella (Simon, 1870) (Araneae, Dictynidae):

BELGIUM • 1 ♂; Tihange, Rue de la Justice, car park near the nuclear power plant; 50°31'57.4"N 5°15'52.6"E; 21.VI.2024; A. & L. Henrard leg.; Beating vegetation; Field number: AH_20240621_01; GenBank (COI): PQ002554; RBINS_IG 34838/01. • 1 ♀; same data as previous; Field number: AH_20240621_02; GenBank (COI): PQ002555; RBINS_IG 34838/02. • 1 ♂; same data as previous; Field number: AH_20240621_03; GenBank (COI): PQ002556; RBINS_IG 34838/03.

DNA-based species identification

Individual DNA was extracted from four legs of each specimen and a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was subsequently amplified using the same methodology as described in HENRARD et al. (2024). Additionally to the LCO1490 and HCO-700ME primer pair (FOLMER et al. 1994; BRETON et al. 2016) For the present investigation was complemented with amplicons obtained using the primers C1-J-1718-spider (5'-GGNGGATTTGGAAATTGRTTRGTTCC-3') and C1-N-2776-spider (5'-GGATAATCAGAATANCGNCGAGG-3') (VINK et al. 2005). Further purification, Sanger sequencing and in silico processing of the raw data were as described in HENRARD et al. (2024). Generated consensus sequences were generated for each 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 sequences were first aligned with all publicly available COI sequences of *Nigma* species downloaded from BOLD and GenBank (<https://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. For this analysis, the final alignment comprised 24 unique COI sequences. Our sequences were trimmed to the universal 658 bp barcoding region of the COI gene to ensure consistency, as all currently available *Nigma* COI sequences from online repositories are limited to this length (see Appendix 1 Table A1). Among these, the COI sequence of *Ajmonia gratiosa* (Simon, 1881) was included, as this species was recently re-transferred from the genus *Nigma* to *Ajmonia* Caporiacco, 1934 (WUNDERLICH 2022). Additionally, the COI sequences of *Dictyna uncinata* and *Dictyna arundinacea* were included to root the NJ tree.

Examination and illustration

Photographs of the specimens 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 (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/>).

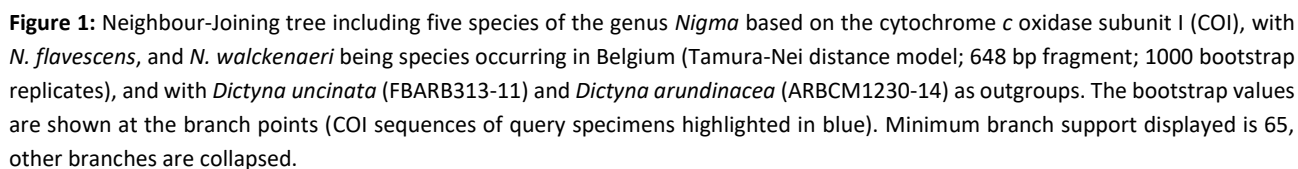
Results

DNA-based analyses

The generated sequences (1229 bp) were unique and deposited on GenBank with accession numbers PQ002554-PQ002556. Eight sites were polymorphic between the three generated sequences ($S = 8$; average number of nucleotide differences, $k: 5.3$), all of them synonymous. Using the Identification System of BOLD, high matches (99.69 - 100%) were obtained with representatives of the species *Nigma puella* (Table 2).

Table 2: Results of the match in BOLD (closest similarity given in %), with collection code and GenBank accession numbers (GB number) of the generated sequences for the specimens collected in Tihange (Belgium).

Collection code	GB number	BOLD similarity (%)
RBINS_IG 34838/01	PQ002554	99.69% <i>Nigma puella</i> (UMAAI2864-22)
RBINS_IG 34838/02	PQ002555	99.85% <i>Nigma puella</i> (private sequence) 99.84% <i>Nigma puella</i> (UMAAI2864-22)
RBINS_IG 34838/03	PQ002556	100% <i>Nigma puella</i> (FBARB1125-16)



Morphological identification

106

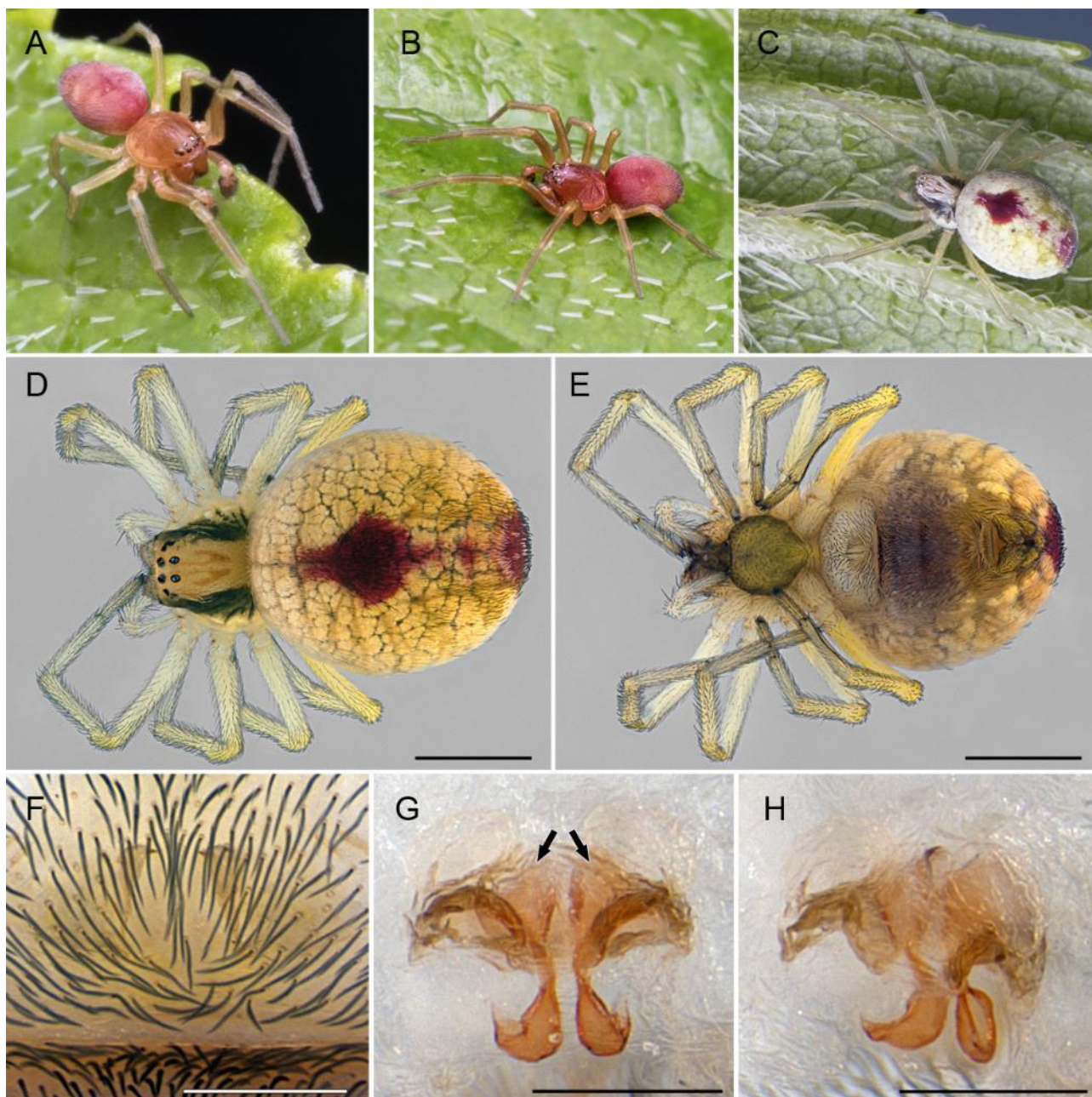


Figure 2: *Nigma puella* (Simon, 1870), specimens collected in Tihange (Belgium). **A-B.** Males (RBINS_IG 34838/01 and RBINS_IG 34838/03). **C-H.** Female (RBINS_IG 34838/02). **A-C.** Habitus *in vivo*. **D.** Habitus in 70% alcohol, dorsal view. **E.** Idem, ventral view. **F.** Epigastric region, ventral view idem. **G.** Vulva, ventral view. The arrows pointing to the large conical sclerite. **H.** Idem, slightly lateral view. Scale bars: D-E = 0.8 mm; E-F = 0.16 mm.

The female genitalia (Fig. 2F-H) are furthermore consistent with illustrations provided in BREITLING (2020: p. 342, fig. 7C) and REHFELDT & CASSAR (2024: p. 22, fig. 7a-b). The males of *Nigma puella* present a reddish pink colouration of the habitus (Figs. 2A-B, 3A-D) and a palpal conformation (Fig. 3E-H) that are very similar to that of *N. flavescens* (Fig. 4A, E). The male of *N. puella* can be distinguished by the broadly rounded basal bulge of the chelicerae (Fig. 3B) (vs. hump with two smaller conical prongs pointing medially in *N. flavescens*, Fig. 4D), and the palp with an embolus course only slightly deviating sub-apically (Fig. 3E-H) (vs. strongly deviating in *N. flavescens*, Fig. 4E) (see also the Key character list hereafter).

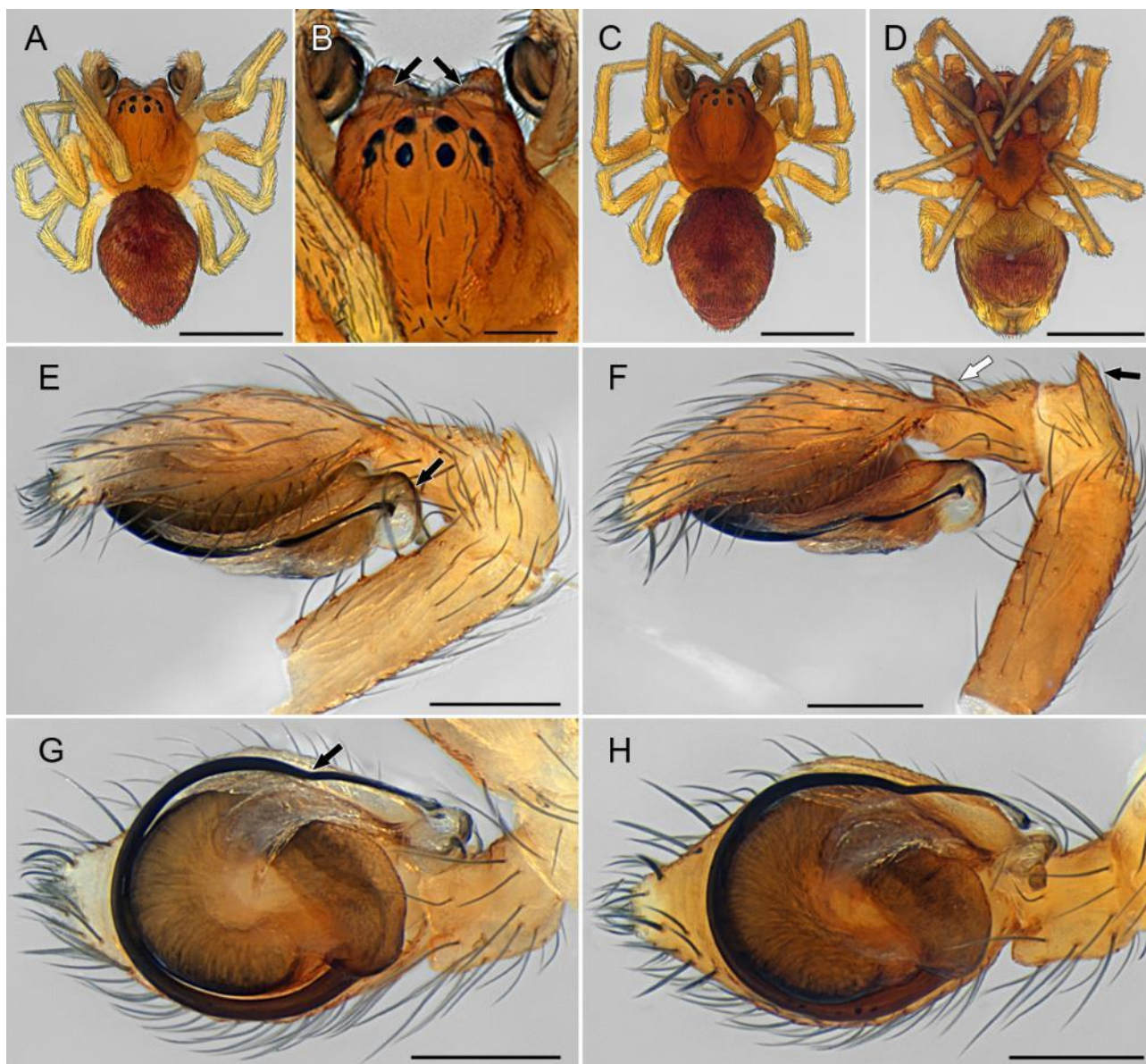


Figure 3: *Nigma puella* (Simon, 1870), male specimens collected in Tihange (Belgium). **A-B, E, G.** Male RBINS_IG 34838/01. **C-D, F, H.** Male RBINS_IG 34838/03. **A, C.** Habitus, dorsal view. **B.** Detail on carapace, dorsal view. The arrows point to the cheliceral bulges. **D.** Habitus, ventral view. **E-F.** Palp, retrolateral view. On E: the black arrow points to the rounded tip of conductor. On F: the black arrow points to the patellar apophysis and white arrow points to the tibial apophysis. **G-H.** Palp, ventral view. On G: the black arrow points to the slight deviation of the embolus course. Scale bars: A-D = 0.8 mm; E-H = 0.16 mm.

Key character list of *Nigma* species found in Belgium

Remark: since only three species occur in Belgium, we do not provide an identification key. Instead, we present a list of the main characteristics for recognising each species. Diagnostic character combinations are highlighted in *italics* and underlining.

Nigma puella (Simon, 1870)

- **Body length.** Male: 2.0–2.8 mm; female: 2.5–3.4 mm.
- **Cephalothorax.** Male (Figs. 2A-B, 3A, C): in living specimen, orange-brown to reddish with shades of pink-brown with lateral margins paler, orange-brown in alcohol; female (Fig. 2C-D): cephalic region yellowish-white, with dense cover of white setae, thoracic region darker and lateral margins white.
- **Abdomen.** Male (Figs. 2A-B, 3A, C): pale reddish pink with faint yellow spots dorso-laterally; female (Fig. 2C-D): whitish, decorated dorsally with reddish markings and greenish patches in-between.
- **Male chelicerae.** Sub-basal bulge broadly rounded.

- **Male pedipalp.** Patella and tibia provided with pointed conical apophysis, embolus course with slight subapical deviation, tip of conductor rounded, with small terminal apophysis (Fig. 3E-H).
- **Female genitalia.** Vulva with large conical sclerites (Fig. 2G-H).
- **Phenology.** Male from April to August; female from April to September (NENTWIG et al. 2024).
- **Habitat.** Cribellate web on leaves of trees and shrubs or in herbs in various environments: gardens, lawns, wasteland, meadow, undergrowth of deciduous forest or pine forest, moors, scrubland, vineyard, pond or river edge, back dune, ... (ROBERTS 1995, 1998; LE PERU 2007).

Nigma flavescens (Walckenaer, 1830)

- **Body length.** Male: 2.25–2.5 mm; female: 2.5– 4.0 mm.
- **Cephalothorax.** Male (Fig. 4A): in living specimen, reddish-orange-brown with lateral margins pale orange, similar in alcohol; female (Fig. 4B-C): variable, reddish-brown to dark green with two rows of white setae converging posteriorly and lateral margins almost white.
- **Abdomen.** Male (Fig. 4A): pale reddish, uniform or with faint pattern showing a faint trident marking anteriorly and chevrons posteriorly; female: variable, similar to the male but more contrasted (Fig. 4B) or whitish yellow with marbling pattern and dark anterior patch (Fig. 4C).
- **Male chelicerae.** Sub-basal hump with two small conical prongs pointing medially (Fig. 4D; LOCKET & MILLIDGE 1951, fig. 27C).

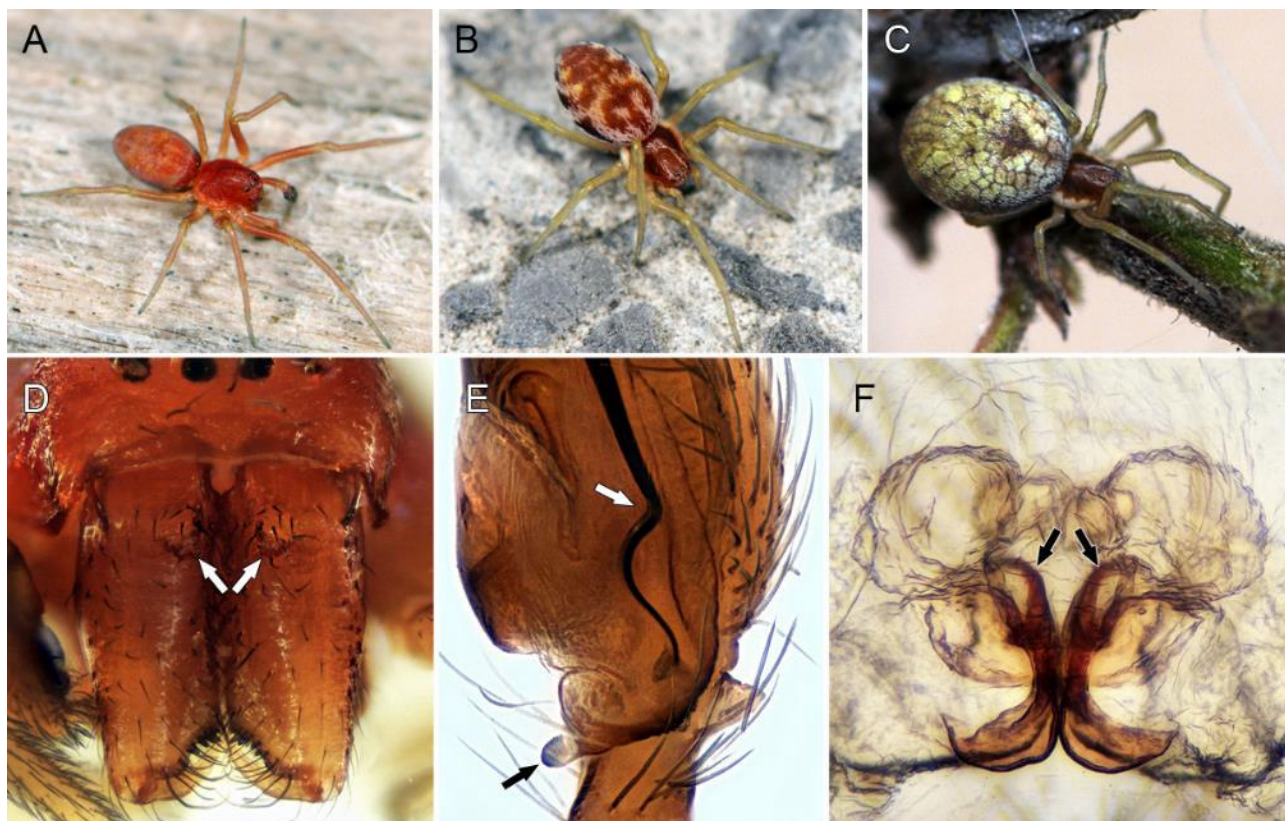


Figure 4: *Nigma flavescens* (Walckenaer, 1830). **A.** Male specimen from Waret-l'Évêque, Belgium (coll. Pierre Oger). **B-C.** Female specimens from Waret-l'Évêque, Belgium (coll. Pierre Oger). **D.** Male from Le Bourget, France (coll. A. Miquet), chelicerae frontal view. White arrows point to the small bulge prongs. **E.** Idem, palp, detail on the conductor, retrolateral view. The white arrow points to the sinuous embolus course, the black arrow points to the stout conductor apophysis. **F.** Female vulva, dorsal view. Black arrows pointing to the conical, curved sclerite. No scales provided. All photos from Pierre Oger.

- **Male pedipalp.** Patella and tibia provided with small, blunt conical apophysis, embolus course strongly sinuous sub-apically, tip of conductor rounded, with stout terminal apophysis (Fig. 4E).
- **Female genitalia.** Vulva with slender, curved conical sclerites (Fig. 4F).
- **Phenology.** Male from April to August; female from April to September (NENTWIG et al. 2024).
- **Habitat.** Especially on leaves of low vegetation, trees and shrubs in forests and forest edges, riparian woodland and riverbanks (ROBERTS 1995, 1998; LE PERU 2007).

Nigma walckenaeri (Roewer, 1951)

- **Body length.** Male: 3.0–4.0 mm; female: 4.0–5.0 mm.
- **Cephalothorax.** Male (Fig. 5A-B): in living specimen, reddish-brown, cephalic region with rows of white setae converging posteriorly, and lateral margins paler, similar in alcohol; female (Fig. 5C): greenish-yellow, cephalic region with rows of white setae converging posteriorly and lateral margins white.
- **Abdomen.** Green to yellowish-green in male and female (Fig. 5A-C).
- **Male chelicerae.** Sub-basal bulge with large conical humps (Fig. 5D).
- **Male pedipalp.** Patella with small triangular apophysis, tibia with blunt apophysis, embolus course with faint subapical deviation, tip of conductor angled, with thin terminal apophysis (Fig. 5E).
- **Female genitalia.** Vulva anteriorly with pair of tube-shaped structures and small, notched conical sclerites, widely separated from each other (Fig. 5F; HENRARD et al. 2024, fig. 2F).
- **Phenology.** Male from August to December; female in May and from August to January (NENTWIG et al. 2024).
- **Habitat.** Frequent on leaves of herbs and bushes, also on the ivy covering the walls of houses. Mostly in gardens and forest edges (ROBERTS 1995, 1998; LE PERU 2007).

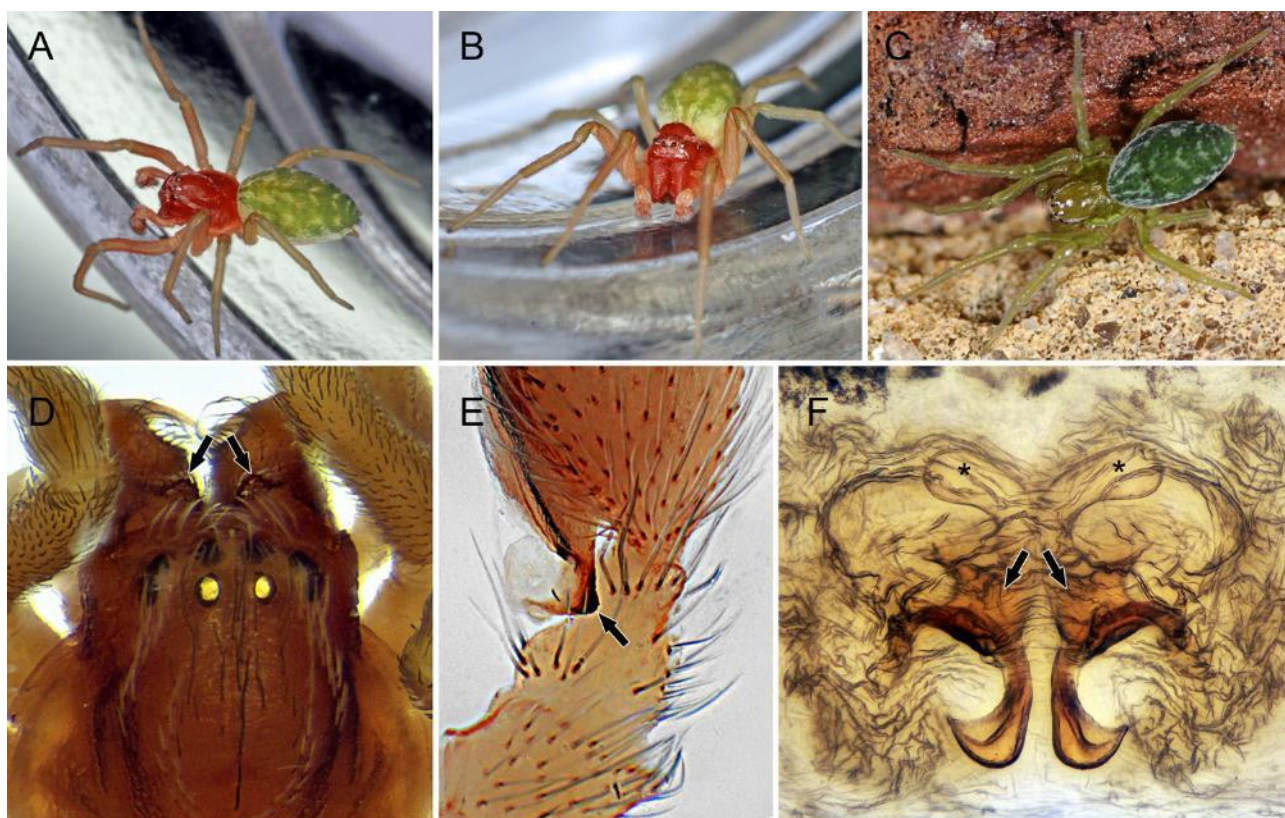


Figure 5: *Nigma walckenaeri* (Roewer, 1951). **A-B, E.** Male specimens from Houx (Yvoir), Belgium (photos & Coll. AH_20100910_01). **C.** Female specimens from Waret-l'Évêque, Belgium (photo & coll. Pierre Oger). **D.** Male from Saint André, France (photo Pierre Oger, coll. G. Melotti), chelicerae dorsal view. The arrows point to the bulge prongs. **E.** Palp, detail on the angled conductor (arrow), retrolateral view. **F.** Female vulva, dorsal view. The arrows point to the conical sclerites, the stars indicate the tube-shaped structure. No scales provided.

Discussion

While much of the Belgian's spider fauna has been well catalogued, including over 700 species (BOSMANS & VAN KEER 2017), ongoing fieldwork and research continue to uncover new species. Even in recent years, previously undocumented species have been identified within Belgium's borders (JANSSEN & CREVECOEUR 2020; HENRARD and DRUMONT 2022; LAMBRECHTS & VAN KEER 2023; HENRARD 2023). Some of these discoveries may represent species that are naturally rare, inhabit previously unexplored regions or habitats. Others could be

the result of shifts in species' geographic ranges, possibly influenced by climate change, while certain non-native species might be introduced through human activities (VAN KEER 2007, 2022).

Given the distribution of the species in the neighbouring countries of Belgium (Fig. 6A-B), it was only a matter of time before the species was reported in our country. *Nigma puella* was first described from Corsica by SIMON in 1870. A few years later, he mentioned its presence in several localities in France, including Honfleur in Normandy (SIMON 1874), which is located less than 300 km from Belgium.

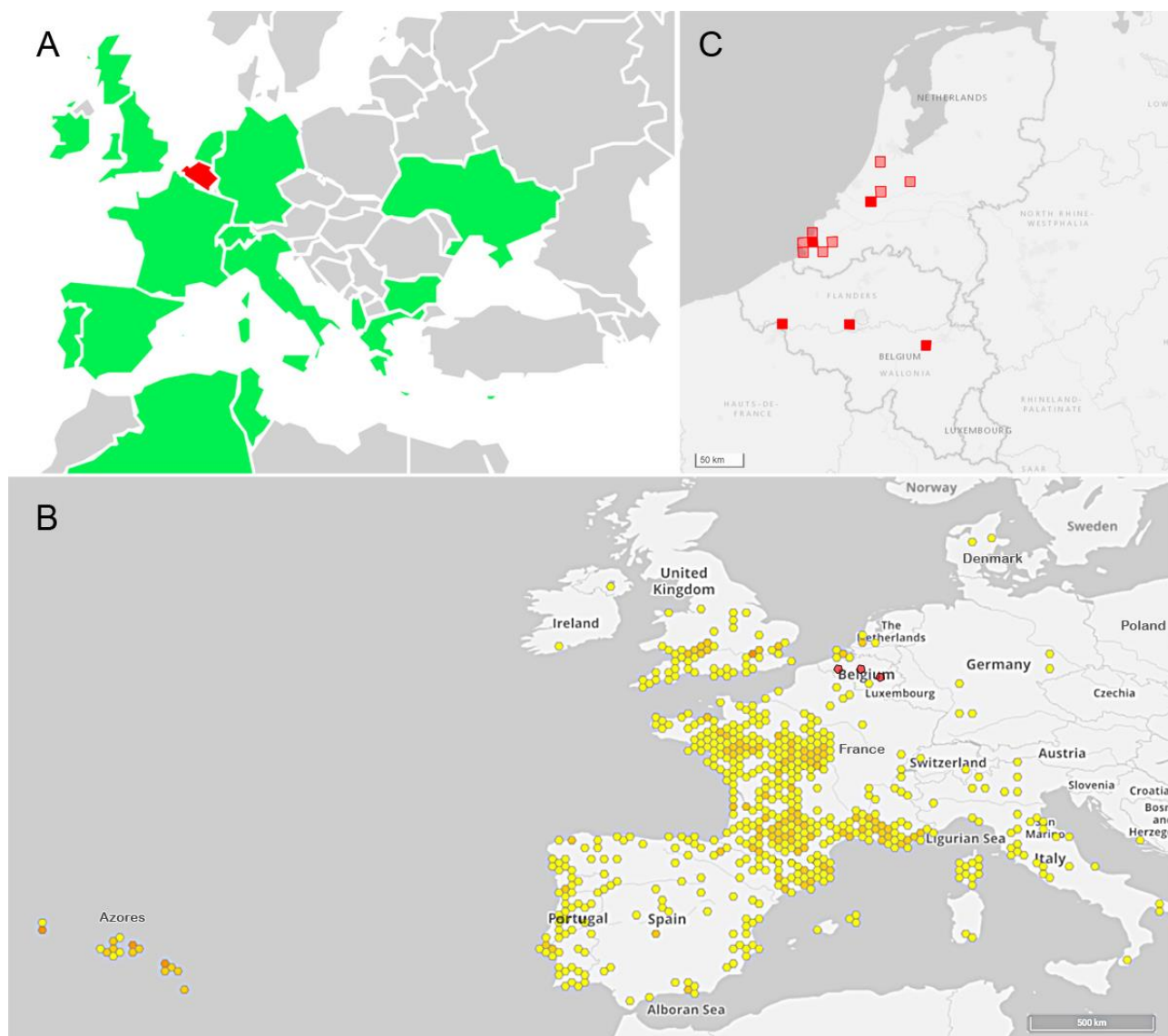


Figure 6: Distribution maps of *Nigma puella* (Simon, 1870). **A-B.** Global distribution: Azores, Madeira, Canary Is., Europe (including Belgium: new records in red), Algeria, Tunisia (A: map after NENTWIG et al. 2024; B: global distribution of records from GBIF 2024, modified). **C.** Detail of the distribution of validated records in Belgium (from OBSERVATIONS.BE / WAARMENINGEN.BE) and Netherlands (from WAARNEMING.NL).

Regarding British records, *Nigma puella* is mentioned in BRISTOWE's *The Comity of Spiders*, Volume II (1941), indicating it was recognized as present in Great Britain at that time. However, the Spider Recording Scheme website notes even earlier records of this species, dating back to 1909 and 1912 (BRITISH SPIDERS 2024; see maps at <https://srs.britishspiders.org.uk/portal.php/p/Time+Series+Maps/s/Nigma%20puella>). It is likely that even older data exists, as confusion between these species has been common, and synonymies have not always been clear (see Simon, 1914, p. 63). PICKARD-CAMBRIDGE (1894, p. 466) and LOCKET & MILLIDGE (1951, p. 64) refer to *Dictyna puella* as synonymous with *Dictyna variabilis* C. L. Koch, 1836, and *Ergatis pallens* Blackwall, 1859 (see also BLACKWALL, 1861), which the WSC currently lists as *Nigma flavescens*. Some

identifications are likely erroneous, such as the individuals from Codford, Wiltshire, found in 1878, whose description given by PICKARD-CAMBRIDGE (1881, p. 466) perfectly matches *N. puella*.

The detection of *N. puella* in Germany occurred much later, with RENNER (1992), and PLATEN *ET AL.* (1995) mentioning the PhD thesis of D. NÄHRIG (1987) as the first record for the country. Finally, the species was only recently recorded for the first time in the Netherlands (VAN DE PUTTE 2018). Since then, multiple sightings have been recorded on various biodiversity monitoring platforms, such as the Global Biodiversity Information Facility (GBIF, 2024) and the Atlas of the European Arachnids (ARACHNOLOGISCHE GESELLSCHAFT 2024). It is also worth noting that the northernmost records were sporadically reported in Denmark, in 1998 and 2022 (ARTER 2024).

Since the first Belgian observation, several records of *N. puella* are now listed on OBSERVATIONS.BE / WAARMENINGEN.BE (<https://observations.be/species/80093>), all representing females identified from photographs. In 2023, it was seen in Tihange (Liège province), Sint-Martens-Lennik (Vlaams-Brabant) and in 2024 in Aalbeke (West-Vlaanderen). One of the latest records in Aalbeke shows a female with spiderlings (<https://observations.be/observation/320411527>), implying that populations are well established in Belgium. These findings suggest a broad distribution in Belgium and the new records align with the species' global distribution. Following the framework proposed by VAN KEER (2007, 2022), when declaring a species as new to Belgium, and in the absence of strong evidence indicating introduction, the species can be considered as part of the Belgian list.

Based on habitus alone, the adult female *N. puella* is easily distinguishable from other closely related species found in Belgium. However, the same is not true for the males, which have a reddish-pink coloration very similar to that of *Nigma flavescens*. Therefore, aside from *Nigma walckenaeri*, which has a distinct red/green colouration, *N. flavescens* or *N. puella* males must be based on microscopic examination of the palpal structure. However, with advancements in computing technology, one can hope that AI-based taxonomic identification applications utilizing deep learning may one day offer a reliable method for identifying males of this species group.

Acknowledgments

The individuals collected in 2023 were found on land that the company Engie dedicated to maintaining biodiversity. We are grateful to Engie for granting us permission to conduct field research on the site and for partially funding both the research and the initial attempt of COI analyses. The second author (GL) would like to thank Jennifer Di Prinzio for facilitating access to the restricted natural site at the Tihange nuclear plant. We thank Johan Michaux and Lise-Marie Pigneur for their valuable help when analysing the first sequences based on juveniles. Paul Selden and Tony Russell-Smith are acknowledged for sharing information and literature on records from Great Britain, and Theo Blick for providing insights regarding Germany. The first author (AH) thanks his daughter Leona Henrard (11 years old) for her help in collecting the spiders in Tihange in June 2024. We are grateful to Wouter Dekoninck for having provided new collection numbers for the specimens deposited at the Royal Institute of Natural Sciences. Pierre Oger is thanked for allowing the use of his pictures, available on his website "Les araignées de Belgique et de France" (<https://arachno.piwigo.com>). We thank Tony Russell-Smith and Jørgen Lissner for reviewing this article. We also thank the editor Bram Vanthournout for his comments and 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).

Author contributions

LB produced the first photograph of *Nigma puella*, posted on Observations.be / Waarmeningen.be and published in Natagora magazine. GL collected the juvenile individuals (08/08/2023) and AH collected the adult specimens (21/06/2024) analysed in this paper. AA made the first attempt at DNA-barcoding on the juveniles collected by GL. NS, MV & AH performed the DNA analyses on the specimens of this study. AH produced photographs, photomicrographs and plates. AH wrote the first draft of the manuscript and edited the revised manuscript. NS wrote the DNA-based species identification and DNA-based analyses sections. GL, LB, MV, NS, AA and KVK commented and edited the revised manuscript.

References

- ARACHNOLOGISCHE GESELLSCHAFT (2024). Atlas of the European Arachnids. *Nigma puella* (Simon, 1870). <https://atlas.arages.de/species/905> (09/2024)
- ARTER (2024). Species recordings from the Danish National portal Arter.dk. Summary for *Nigma puella*. <https://arter.dk/taxa/taxon/details/0f755635-a1aa-45be-bbcc-aefb01514c07> (Nov. 2024)
- ASTRIN, J.J., HÖFER, H., SPELDA, J., HOLSTEIN, J., BAYER, S., HENDRICH, L., HUBER, B.A., KIELHORN, K.H., KRAMMER, H.J., LEMKE, M., MONJE, J.C., MORINIÈRE, J., RULIK, B., PETERSEN, M., JANSSEN, H., MUSTER, C. (2016). Towards a DNA Barcode Reference Database for Spiders and Harvestmen of Germany. *PLoS One*. **Sep 28**;11(9):e0162624. <https://doi.org/10.1371/journal.pone.0162624>
- BORK, R. J., (2015). Primer efficacy in the DNA barcoding of spiders. Honors Program Theses. University of Northern Iowa, pp. 17-18.
- BOSMANS, R. & VAN KEER, K. (2017). Een herziene soortenlijst van de Belgische spinnen (Araneae). *Nieuwsbrief van de Belgische Arachnologische Vereniging* **32**: 39-69.
- BREITLING, R. (2020). South European spiders from the Duffey collection in the Manchester Museum (Arachnida: Araneae). *Arachnology* **18**(4): 333-362. <https://doi.org/10.13156/arac.2020.18.4.333>
- BRISTOWE, W. S. (1941). *The comity of spiders*. Volume II. Ray Society, London, pp. 229-560, pl. 20-22.
- BRITISH SPIDERS (2024). Spider and Harvestman Recording Scheme website the national recording schemes for spiders and harvestmen in Britain. Summary for *Nigma puella* (Araneae). <https://srs.britishspiders.org.uk/portal.php/p/Summary/s/Nigma%20puella> (09/2024)
- BRETON, S., BURGER, G., STEWART, D.T., BLIER, P.U. (2006). Comparative analysis of gender associated complete mitochondrial genomes in marine mussels (*Mytilus* spp.). *Genetics* **172**: 1107-1119.
- CASTELLUCCI, F., SCHARFF, N. & LUCHETTI, A. (2023). Molecular systematics and phylogenetics of the spider genus *Mastigusa* Menge, 1854 (Araneae, Cybaeidae). *Molecular Phylogenetics and Evolution* **186**(107833): 1-9. <https://doi.org/10.1016/j.ympev.2023.107833>
- CASTELLUCCI, F., LUCHETTI, A. & SCHARFF, N. (2024). Enigmatic and extravagant genitalia in the spider genus *Mastigusa* (Araneae, Cybaeidae) – a taxonomical revision. *Invertebrate Systematics* **38**(IS23047): 1-27. <https://doi.org/10.1071/IS23047>
- FOLMER, O., BLACK, M., HOEH, W., LUTZ, R., VRIJENHOEK, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**(5):294-9.
- GORNEAU, J. A., CREWS, S. C., CALA-RIQUELME, F., MONTANA, K. O., SPAGNA, J. C., BALLARIN, F., ALMEIDA-SILVA, L. M. & ESPOSITO, L. A. (2023). Webs of intrigue: museum genomics elucidate relationships of the marronoid spider clade (Araneae). *Insect Systematics and Diversity* **7**(5, 5): 1-18. <https://doi.org/10.1093/isd/ixad021>
- HENRARD, A. & DRUMONT, A. (2022). Updated status of *Saitis barbipes* (Simon, 1868) (Araneae, Salticidae) in Belgium. *Journal of the Belgian Arachnological Society* **37**(1): 1-11.
- HENRARD, A., BAERT, L., DE SMEDT, P., GARDINI, G., VANHERCKE, L., JOCQUÉ, R., OGER, P., KEKENBOSCH, R., VAN NIEUWENHOVE, C., LOCK, K. & DRUMONT, A. (2022). On the arachnofauna of the Jean Massart botanical garden (Brussels-Capital Region, Belgium). *Journal of the Belgian Arachnological Society* **37**(2): 122-137.
- HENRARD, A. (2023). On some introduced species in Belgium: new spiders detected and new records. *Journal of the Belgian Arachnological Society* **38**(2): 71-83.
- HENRARD, A., KRATZ, F., SMITZ, N., BAERT, L., DRUMONT, A., VAN KEER, K. (2024). On the female specimen *Nigma walckenaeri* (Roewer, 1951) misidentified as *Nigma puella* (Simon, 1870) – Erratum for "Henrard, A., Baert, L., De Smedt, P., Gardini, G., Vanhercke, L., Jocqué, R., Oger, P., Kekenbosch, R., Van Nieuwenhove, C., Lock, K. & Drumont, A. (2022). On the arachnofauna of the Jean Massart botanical garden (Brussels-Capital Region, Belgium). *Journal of the Belgian Arachnological Society* 37(2): 122-137". *Journal of the Belgian Arachnological Society* **39**(2): 153-160.
- JANSSEN, M. & CREVECOEUR, L. (2020). *Midia midas* (Simon, 1884) (Araneae, Linyphiidae), a species new to the Belgian spider fauna. *Nieuwsbrief van de Belgische Arachnologische Vereniging* **35**(3): 83-88.
- LAMBRECHTS, J. & VAN KEER, J. (2023). *Nurscia* spec. (Titanoecidae) nieuw voor België, in de bermen van de Brusselse Ring. *Journal of the Belgian Arachnological Society* Volume **38**(1): 10-16.
- LE PERU, B. (2007). Catalogue et répartition des araignées de France. *Revue Arachnologique* **16**: 1-468.
- LOCKET, G. H. & MILLIDGE, A. F. (1951). *British spiders. Vol. I*. Ray Society, London, 310 pp.
- NÄHRIG, D. (1987). Spinnenfauna der oberen Streuschicht von Hecken in Flurbereinigungsgebieten. Diss. Univ. Heidelberg. 222 S. u. Anh.
- NENTWIG, W., BLICK, T., BOSMANS, R., GLOOR, D., HÄNGGI, A. & KROPF, C. (2024) Spiders of Europe. Version 06.2024. Online at <https://www.araneae.nmbe.ch>, accessed on July 2024. <https://doi.org/10.24436/1>
- OBSERVATIONS.BE / WAARMENINGEN.BE (2024), Natagora, Natuurpunt and Fondation "Observation International". <https://observations.be/> <https://waarmeningen.be> (09/2024).
- ONO, H. & OGATA, K. (2018). *Spiders of Japan: their natural history and diversity*. Tokai University Press, Kanagawa, 713 pp.
- PICKARD-CAMBRIDGE, F. O. (1881). The spiders of Dorset, with an appendix containing short descriptions of those British species not yet found in Dorsetshire. *Proceedings of the Dorset Natural History and Antiquarian Field Club* **2**: 237-625.
- PICKARD-CAMBRIDGE, F. O. (1894). Part III. Systematics list of GENERA and SPECIES with synonyms of the latter. in Robson, J.E. & Hartlepool, F.E.S. *The British Naturalist: an illustrated magazine of natural history*, v.3 &n.s. v.1-12 (1893-1894), London, pp. 262-263. Available at <https://www.biodiversitylibrary.org/item/172443#page/7/mode/1up> (09/2024)
- REHFELDT, S. & CASSAR, T. (2024). A contribution to the arachnofauna (Arachnida: Araneae, Opiliones) of the Maltese Islands, with two new records for Europe. *Arachnologische Mitteilungen* **67**: 18-47. <https://doi.org/10.30963/aramit6704>

- RENNER, F. (1992). Liste der Spinnen Baden-Württembergs (Araneae). Teil 2: Liste der Spinnen Baden-Württembergs excl. Linyphiidae, Nesticiidae, Theridiidae, Anapidae und Mysmenidae. *Arachnologische Mitteilungen* **4**: 21-55. <https://doi.org/10.5431/aramit0402>
- ROBERTS, M. J. (1995). *Collins Field Guide: Spiders of Britain & Northern Europe*. HarperCollins, London, 383 pp.
- ROBERTS, M. J. (1998). *Spinnengids*. Tirion, Baarn, Netherlands, 397 pp.
- SIMON, E. (1870). Aranéides nouveaux ou peu connus du midi de l'Europe. *Mémoires de la Société Royale des Sciences de Liège* (2) **3**: 271-358.
- SIMON, E. (1874). *Les arachnides de France*. Paris 1, 1-272.
- VAN DE PUTTE, F. (2018). *Nigma puella* (Simon, 1870), het Bont kaardertje, nieuw voor Nederland (Araneae, Dictynidae). *Nieuwsbrief Spined* **37**: 2-4
- VAN KEER, K. (2007). Exotic spiders (Araneae): Verified reports from Belgium of imported species (1976-2006) and some notes on apparent neozoan invasive species. *Nieuwsbrief van de Belgische Arachnologische Vereniging* **22**(2): 50
- VAN KEER, K. (2022). Nieuwe spinnen voor de Belgische fauna: naar een consequent gebruik van standaarden voor soortenstatus. *Journal of the Belgian Arachnological Society* **37**(2): 108-118.
- VINK C.J., THOMAS S.M., PAQUIN P., HAYASHI C.Y., HEDIN M. (2005) The effects of preservatives and temperatures on arachnid DNA. *Invertebrate Systematics* **19**(2): 99-104. <https://doi.org/10.1071/IS04039>
- WHEELER, W. C., CODDINGTON, J. A., CROWLEY, L. M., DIMITROV, D., GOLOBOFF, P. A., GRISWOLD, C. E., HORMIGA, G., PRENDINI, L., RAMÍREZ, M. J., SIERWALD, P., ALMEIDA-SILVA, L. M., ÁLVAREZ-PADILLA, F., ARNEDO, M. A., BENAVIDES, L. R., BENJAMIN, S. P., BOND, J. E., GRISMADO, C. J., HASAN, E., HEDIN, M., IZQUIERDO, M. A., LABARQUE, F. M., LEDFORD, J., LOPARDO, L., MADDISON, W. P., MILLER, J. A., PIACENTINI, L. N., PLATNICK, N. I., POLOTOW, D., SILVA-DÁVILA, D., SCHARFF, N., SZÜTS, T., UBICK, D., VINK, C., WOOD, H. M. & ZHANG, J. X. (2017). The spider tree of life: phylogeny of Araneae based on target-gene analyses from an extensive taxon sampling. *Cladistics* **33**(6): 576-616. <https://doi.org/10.1111/cla.12182>
- WUNDERLICH, J. (2022). Some spiders (Araneae) of the Western Palearctic. *Beiträge zur Araneologie* **15**: 4-78, 199.
- WAARNEMING.NL (2024), Natagora, Natuurpunt et la Fondation "Observation International". <https://waarneming.nl/> (09/2024).
- WORLD SPIDER CATALOG (2024). World Spider Catalog. Version {version number}. Natural History Museum Bern, online at <http://wsc.nmbe.ch>, accessed on 07/2024. <https://doi.org/10.24436/2>

Appendix 1. List of COI sequences

Table A1: 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	/	Henrard et al. 2024
<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
Shade	RBINS_IG 34838/01	PQ002554	/	This study
	RBINS_IG 34838/02	PQ002555	/	This study
	RBINS_IG 34838/03	PQ002556	/	This study
<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