An addition to the list of wild bee fauna of Luxembourg: *Nomada kohli* Schmiedeknecht, 1882 (Hymenoptera, Apidae), with a list of the species of the genus *Nomada* Scopoli, 1770 recorded in the country

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Abstract. The cleptoparasitic bee *Nomada kohli* Schmiedeknecht, 1882 has been recorded for the first time in Luxembourg during the first year of the recently launched national monitoring scheme of wild pollinators (MONIPOL). A single male specimen was captured the 6th of August 2019 at the locality of Blaschette, on a roadside along pastures. We can thus add a new record to the list of 39 species of *Nomada* previously recorded in the country, for a total of 40 species.

Key words. *Nomada kohli*, wild bee, cuckoo bee, Apidae, DNA barcodes, Luxembourg.

1. Introduction

Wild pollinators are declining at local, regional, and global scales, in both diversity and abundance, primarily as consequence of human activities (Biesmeijer et al. 2006, Potts et al. 2015, 2016). While declines have been well documented in some countries of north-west Europe, major knowledge gaps still exist across taxa and geographic regions within Europe (Potts et al. 2021), including Luxembourg.

Considering its small size, Luxembourg has a relatively rich wild bee fauna, with 346 recorded species compared to ca. 400 in Belgium and 366 in the Netherlands (Schneider 2018, Weigand & Herrera-Mesías 2020, Herrera-Mesías & Weigand 2021). However, the bee fauna of the country has not been evenly and systematically sampled at the national extent. Various initiatives are being developed to update wild bee species lists and provide detailed information regarding their distribution, as well as to detect potentially species-rich areas (IEEP 2019).

In addition to inventorying and documenting species distribution, monitoring changes in species occupancy, richness and abundance is essential to understand the impacts of environmental change and the efficacy of conservation actions. Since 2019, the Luxemburg Institute of Science and Technology (LIST) has been implementing the national monitoring scheme of wild pollinators (MONIPOL). The aim of MONIPOL is to systematically obtain population trend data for a broad taxonomic range of wild pollinating insects, such as wild bees, in a set of representative sites across Luxembourg's

wider countryside, using a sampling design and survey methods compatible with the EU Pollinator Monitoring Scheme (Potts et al. 2021). These data are needed to assess how wild pollinator populations change at the national extent in the long term. As MONIPOL aims to collect data with sampling effort repeated at the same places over time, it is likely that new species will continue to be added to the current bee fauna in the coming years. Here, we document the first of such new records.

The genus *Nomada* (body length: 4 to 17 mm) comprises bees with a wasp-like appearance, often with bold bands of yellow, red, orange and black colours and limited pilosity (Michez et al. 2019). All the species from this genus are cleptoparasites ("cuckoo bees"): unlike other bees, females do not build their own nests or harvest floral resources for their brood, but roam around looking for nests of their ground-dwelling host (often limited to one bee species) to lay an egg in an unsealed nest cell. The *Nomada* larva then destroys the egg or the larva of the host bee and feeds on it and on its food reserves. While larvae depend exclusively on the food resources harvested by their host, adults may use a wide range of flowers to feed on nectar and pollen (Michez et al. 2019). *Nomada* mainly parasitizes bees of the genera *Andrena, Eucera, Lasioglossum, Melitta* and *Panurgus* (Dufrêne et al. 2014).

Nomada is the most diversified genus of cuckoo bees worldwide, with most species distributed throughout the Holarctic region (Michez et al. 2019). According to Smit (2018), 208 species have been recorded in Europe, and between 800 to 850 species are described globally (Falk 2015, Michez et al. 2019). Thirty-nine species of *Nomada* had been previously recorded in the Grand-Duchy of Luxembourg (Table 1), with an additional species (*Nomada similis*) recorded in Steinbach, only 1.4 km from the border, in Belgium. The database of the National Museum of Natural History Luxembourg holds records from 34 species that were collected from the country between 1955 and 2020 (MNHNL 2021).

2. Material and Methods

2.1. Sampling strategy and methods

MONIPOL has adopted two standardised methods for the monitoring of wild pollinators at the national extent, namely bumblebee walks (bumblebee observations along a 2.5 km-long transect) and pan-trapping. Systematic surveys are conducted in a set of approximately 30 1×1km squares randomly selected from each of 10 strata representing the main ecological regions and main habitat types in Luxembourg in proportion to their surface (Titeux et al. 2009). In each of these squares, pan-trapping is conducted in five separate sites along the bumblebee transect, on four separate occasions per year between April and September. Pan-traps consist of a set of 3 plastic bowls of three colours: white, UV

Table 1. List of *Nomada* species recorded in Luxembourg. With the new record of *Nomada kohli* (in bold), the list comprises 40 species. [1] No specimen deposited in the collection of the MNHNL

Species	Source				
	Cungs et al. (2007)	Feitz et al. (2001)	Feitz et al. (2006)	Rasmont et al. (2017)	MNHNL (2021)
Nomada alboguttata			x	x	x
Nomada armata	x	x		x	x
Nomada bifasciata	x		x	x	x
Nomada braunsiana				x	x
Nomada castellana				x	x

Table 1. (continued)

Species	Source				
	Cungs et al. (2007)	Feitz et al. (2001)	Feitz et al. (2006)	Rasmont et al. (2017)	MNHNL (2021)
Nomada conjungens				x	x
Nomada distinguenda	x				x
Nomada emarginata [1]					x
Nomada fabriciana	x		x	x	x
Nomada facilis				x	
Nomada ferruginata		x	x	x	x
Nomada flava	x		x	x	x
Nomada flavoguttata	x		x	x	x
Nomada flavopicta			x	x	
Nomada fucata	x		x	x	x
Nomada fulvicornis	x			x	x
Nomada furva					x
Nomada fuscicornis				x	x
Nomada goodeniana				x	x
Nomada insignipes				x	
Nomada integra	x				
Nomada kohli					
Nomada lathburiana	x		x	x	x
Nomada leucophthalma	x	x	x	x	x
Nomada marshamella	x		x	x	x
Nomada melathoracica		x		x	x
Nomada obscura				x	
Nomada obtusifrons				x	x
Nomada panzeri				x	x
Nomada rhenana				x	x
Nomada roberjeotiana [1]					x
Nomada ruficornis				x	x
Nomada rufipes				x	x
Nomada sexfasciata	x		x		x
Nomada sheppardana	x			x	x
Nomada signata	x	x	x	x	x
Nomada stigma		x		x	x
Nomada striata	x			x	x
Nomada succincta	x		x	x	x
Nomada zonata	x	x		x	x



Fig. 1. Pan-trap set used in the national monitoring scheme of wild pollinators (MONIPOL), composed of three UV-painted bowls, placed on their support at a height equal or slightly higher than the average vegetation height.

blue, and UV yellow, which are placed on stakes at vegetation height to mimic flowers (Fig. 1). The bowls are half-filled with water, to which a couple of drops of liquid soap are added to reduce surface tension. The pan-traps are recovered the following day, making sure they stayed in place for 24 hours. With this survey effort, the average number of bees caught per pan-trap set (approximately 5 specimens) is fairly low and thus unlikely to have a detrimental effect on local populations. All insect specimens are collected and frozen or stored in 70 % non-methylated ethanol until processed for identification. As the amount of floral resources around the pan-traps greatly influences the catch (Potts et al. 2021), the observer makes a floristic survey within a two-meter-radius around the pan-trap.

2.2. Specimen identification

The specimen was determined morphologically following the keys of Amiet et al. (2007), Scheuchl (2000) and Smit (2018). Photographs of all key traits needed for determination were taken with the aid of a Keyence VHX-S660E digital microscope at the National Museum of Natural History Luxembourg.

The specimen was also identified by traditional Sanger sequencing. DNA was extracted from a single front leg of the dry-pinned specimen. A 421 bp region within the mitochondrial cytochrome c oxidase subunit I gene (COI) was amplified using the BF2/BR2 primer pair (Elbrecht & Leese 2017). For DNA extraction, polymerase chain reaction (PCR), PCR purification and Sanger sequencing, the laboratory protocols described in Weigand & Herrera-Mesías (2020) were used. PCR thermal cycling was based on Elbrecht & Steinke (2019): initial denaturation step at 94°C for 5 minutes, 34 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 48.8°C, extension at 65°C for 50 seconds, followed by a final extension for 5 minutes also at 65°C.

Successful PCR products were precipitated with ethanol and forward and reverse strands sequenced using the Big Dye Terminator chemistry (Applied Biosystems, Carlsbad, CA, USA) on an ABI 3730 capillaryDNA automated sequencer (Applied Biosystems).

Geneious Prime 2019.1.1 (Kearse et al. 2012) was used to inspect the chromatograms and produce the fasta files containing the final sequence. Finally, the individual COI DNA barcodes were compared against sequences stored in the Barcode of Life Data system (BOLD; Ratnasingham & Hebert 2007).

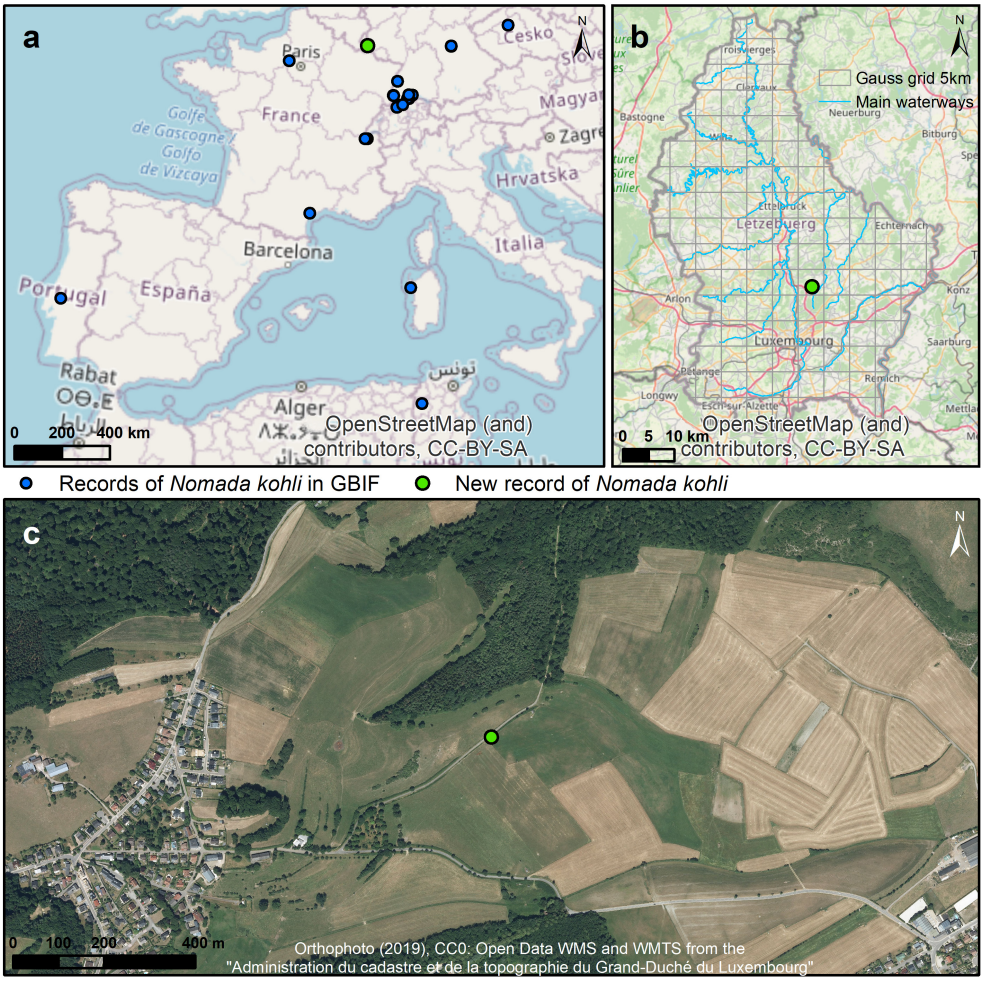


Fig. 2. a, Records of *Nomada kohli* in the GBIF database (GBIF.org 2021). b, Location of the new record of *N. kohli* in Luxembourg. c, Aerial view of the landscape around the new record, near Blaschette. All maps are projected in Luxembourg 1930 Gauss.

3. Results

3.1. Sampling site characteristics

The voucher (MNHNL100058, BOLD-ID: MNPL001-21) is a single male specimen of *Nomada kohli*, caught in the white bowl of the three-colour pan-trap set, representing the first record of this species in Luxembourg. The pan-trap was placed on the 5th of August 2019 at 13:00 hrs and collected on the 6th of August 2019 at 11:00 am, in the locality of Blaschette (Gauss-Luxembourg: 80755 E, 85585 N; Lon/Lat WGS84: 6.17859°E, 49.7048°N; Fig. 2b). The pan-trap was set on a roadside along pastures, with the presence of hedge bindweed (*Calystegia sepium* (L.) R.Br. 1810), creeping thistle (*Cirsium arvense* (L.) Scop. 1772), field bindweed (*Convolvulus arvensis* L. 1753), red clover (*Trifolium pratense* L. 1753), and thymes (*Thymus* spp.). On the day of capture, only one individual of *C. arvensis* and one of *C. arvense* were in flower within a two-meter-radius around the trap. The surrounding landscape is mainly agricultural with a dominance of pastures and some woods, the closest being about 80 m from the pan-trap set (Fig. 2c). Other bee species cap-

tured in the same pan-trap station between July and September 2019 were *Halictus tumulorum* (Linnaeus, 1758), *Lasioglossum glabriusculum* (Morawitz, 1872), *Lasioglossum leucozonium* (Schrank, 1781), *Lasioglossum pauxillum* (Schenck, 1853) and *Lasioglossum puncticolle* (Morawitz, 1872).

3.2 Systematic account

Scientific name: *Nomada kohli* Schmiedeknecht, 1882
Common names: Nomade de Kohl (French), Kohls Wespenbiene (German)

Family: Apidae Latreille, 1802
Subfamily: Nomadinae Latreille, 1802

Genus: *Nomada* Scopoli, 1770

Morphological species diagnosis (male), following Amiet et al. (2007) and Scheuchl (2000): A small (5-7cm) dark coloured *Nomada* presenting sparse yellow markings on the sides of a dark coloured abdomen (Fig. 3a,b). Mandibles are simple, with the apex terminated in a single pointed end (Fig. 3c). Labrum is yellow, dark in the middle, more or less densely punctured and with three small teeth protruding in the

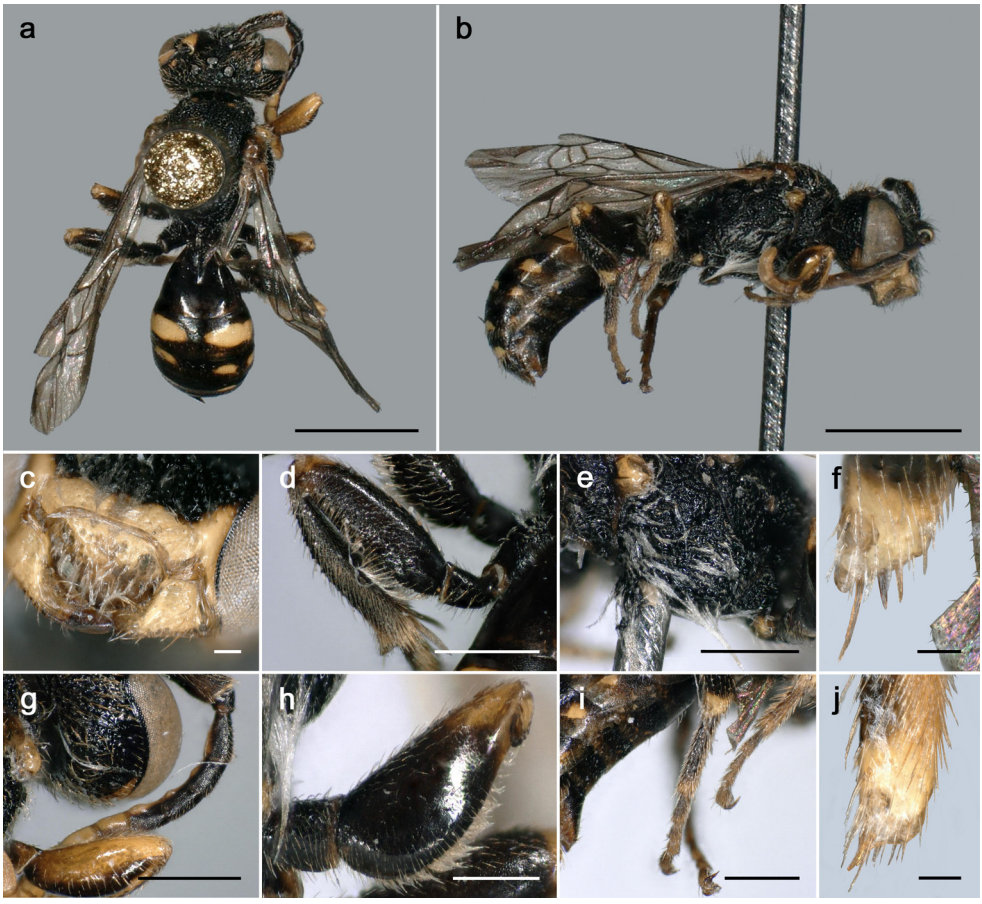


Fig. 3. *Nomada kohli* Schmiedeknecht, 1882 (Blaschette, MNHNL100058, BOLD-ID: MNPL001-21, one front leg was used for DNA extraction). a, Dorsal view; b, Lateral view; c, Mandibles and labrum; d, Hind femur and trochanter; e, Mesopleuron, showing the white hairs between the coxae; f, Spines at the end of the hind tibia; g, Antennal flagellar segments; h, Femur 2; i, Hind metatarsus; j, Middle tibia, terminated in a spine. Scale bars: a, b = 1 mm; d, e, g, i = 0.5 mm; h = 0.25 mm; c, f, j = 100 µm. Photos: MNHNL. Background edited using GIMP 2.8.22 (The GIMP Development Team 2020).

middle (Fig. 3c). The hind femur presents a hair fringe that does not extend into the trochanter (Fig. 3d). The mesopleuron presents long, white hairs between the coxae (Fig. 3e). The hind tibiae are blunt, at the end with hairs and two to three pale spines (Fig. 3f). The antennal segment 3 is longer than 4 and segments 8 to 12 are longer than broader, with segments 4 to 6 presenting distinctly pronounced nodules (Fig. 3g). The femur 2 is strongly broadened (Fig. 3h). The hind metatarsus is straight, not widened and without conspicuous hair fringes (Fig. 3i). The middle tibia extends into conspicuously long, pointed spine that is a key diagnostic trait for the species (Fig. 3j).

3.3. Molecular identification

The taxonomic annotation is partially supported by DNA barcoding data. When the sequence comparison included only published COI records from BOLD and GenBank, the best percentage of sequence identity was achieved with *N. kohli* (94.96 %, BOLD-ID: FBAPB236-09, GenBank-ID: HQ948035, collected in Tunisia). This specimen corresponds to the only barcode of *N. kohli* available in the public database.

When all BOLD records were considered, the best percentages of sequence identity were achieved with an unspecified *Nomada* (97.6%, BOLD-ID: GMIHJ218-19, private record, collected in Israel) and with a specimen labelled as *Nomada distinguenda* (97.6%, BOLD-ID: TDAAT285-19, private record, collected in Austria). However, this last case may correspond to a misidentification, as discussed below.

4. Discussion

Both the morphological and molecular identification support the annotation of *N. kohli* for the Luxembourg specimen. Even though an intraspecific genetic distance of 5.04% was found between this specimen and the Tunisian *N. kohli*, the status of the remaining records and the fact that the mean and maximum intraspecific distances of *N. kohli* are yet to be determined (Appendix S5 in Schmidt et al. 2015) make this annotation the most likely among the available options. Our record thus adds to the 39 species of *Nomada* previously recorded in the country, for a total of 40 species.

Regarding the high percentage match of the Luxembourg *N. kohli* DNA sequence with that of the private record of *N. distinguenda* from Austria (BOLD-ID: TDAAT285-19), it is important to note that currently, there are 10 public records of *N. distinguenda* published in the BOLD database, which cluster together into a single Barcode Index Number (BIN). Yet, none of these sequences matched the Luxembourg specimen. Moreover, when tree-based identification is performed using BOLD sequences (neighbour-joining tree, Kimura 2 Parameter), the cluster containing the public records of *N. distinguenda* is completely absent from the tree (Fig. 4). Given that *N. kohli* and *N. distinguenda* are visually similar and share several morphological traits, potential misidentifications of specimens under suboptimal conditions are likely to happen. If possible, a taxonomic re-evaluation of the Austrian specimen labelled as *N. distinguenda* using a digital microscope might provide further information on this aspect.

N. kohli has a West-Palaearctic distribution. It is widespread in southern Europe and more sporadic in central Europe (Fig. 1a). It has been recorded in some countries neighbouring Luxembourg, such as France (Dufrêne et al. 2014) and Germany (Westrich et al. 2011), but not in Belgium. The species is (partially) bivoltine. In Switzerland, the first generation occurs from late April to late June and the second from late July to late August, but most records are from mid-June to mid-August (Amiet et al. 2007). It overwinters as an adult. This species is not threatened (i.e., Least Concern) at the European extent (Nieto et al. 2014) but is highly threatened in Germany (Westrich et al. 2011), where its current population status is rare, showing a strong long-term population decline.

N. kohli uses *L. puncticolle* (Morawitz, 1872) as a host (Amiet et al. 2007, Scheuchl 2000, Smit 2018). This is a small, possibly eusocial ground-nesting bee (4.5-5.5 mm) that occupies various habitats and feeds on

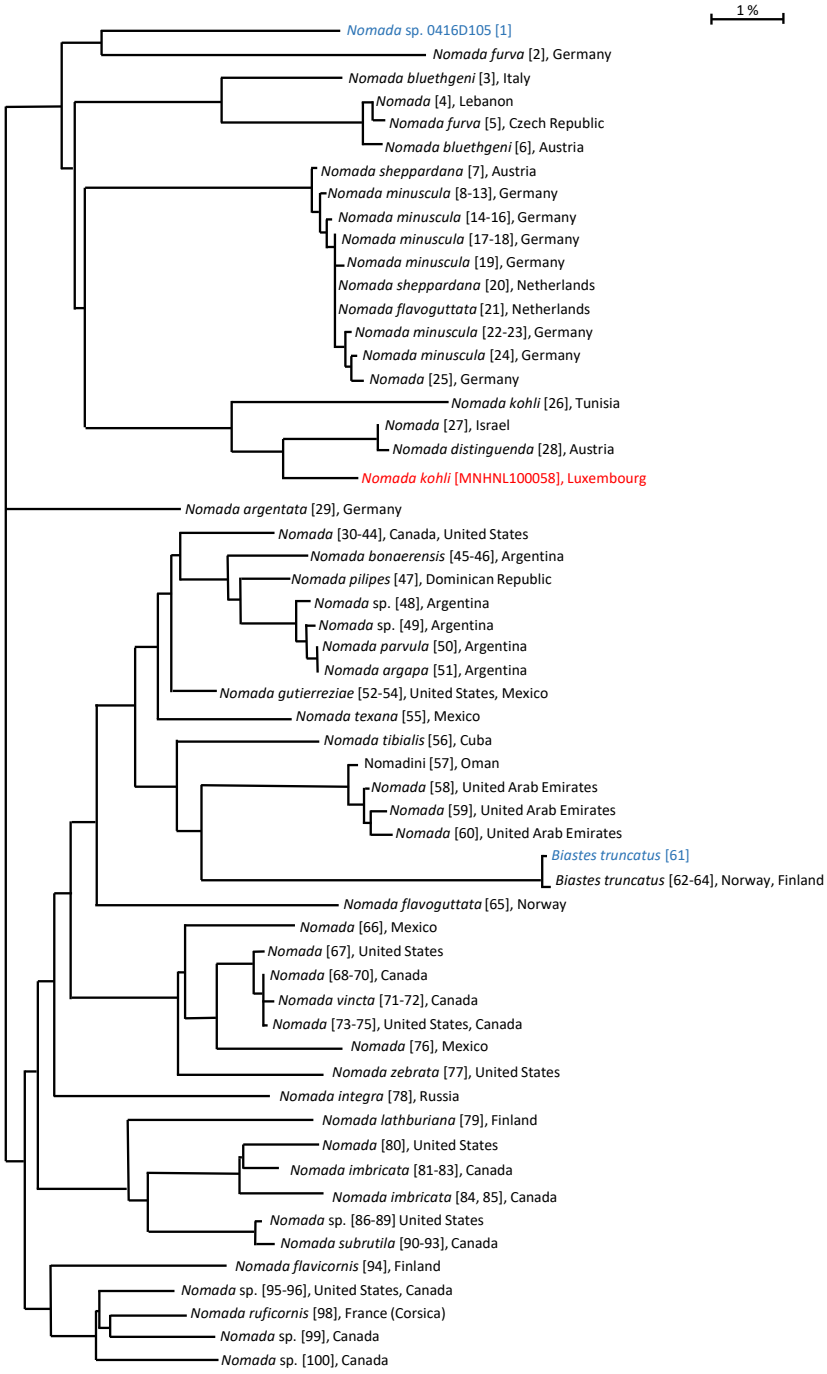


Fig. 4. Overview BOLD TaxonID Tree of COI sequence comparison of the voucher specimen of *Nomada kohli* MNHNL100058 (in red) with *Nomada* sequences in the Barcode of Life Data system (BOLD; Ratnasingham & Hebert 2007). BOLD sequences in black. GenBank sequences in blue. Data retrieved on 9th December 2020. Data type: Nucleotide. Distance Model: Kimura 2 Parameter. Image edited using GIMP 2.8.22 (The GIMP Development Team 2020).

various floral resources (Falk 2015). As *L. puncticolle* was pan-trapped in the same sampling site as the new *N. kohli* record, further research in the region would be useful to determine if this isolated finding is an indication of a previously unknown population of *N. kohli* established in Luxembourg's territory.

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