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Molecular identification of the potentially forensically relevant cluster flies *Pollenia rudis* (Fabricius) and *Pollenia vagabunda* (Meigen) (Diptera: Polleniidae) — non-recorded species in Algeria

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ABSTRACT

Cluster flies are represented by the genus *Pollenia* Robineau-Desvoidy, 1830 of the family Polleniidae Brauer and Bergenstamm, 1889. Their larvae are known to be internal parasites or predators of earthworms. Herein, we report for the first time the occurrence of the cluster flies *Pollenia rudis* Fabricius, 1794 and *Pollenia vagabunda* (Meigen, 1826) (Diptera: Polleniidae) on carcasses in Algeria and identify them through DNA barcoding. A region of the mitochondrial cytochrome c oxidase I gene (*COI*) was amplified and sequenced. Genetic distances were determined. A phylogenetic tree was constructed with the maximum parsimony method using 10 000 bootstrap replicates. A total number of 157 adults of *P. rudis* were collected together with 325 adults of *Pollenia vagabunda*. The occurrence of *Pollenia* on animal carcasses does not seem to be correlated with a particular stage of decomposition. All the sequences were correctly identified using the BLASTn tool from the GenBank database and the BOLD identification engine. Intra- and interspecific sequence divergence values were less than 1% and greater than 3%, respectively. *COI* barcodes obtained from this study were robust enough to identify and distinguish unambiguously between *P. rudis* and *P. vagabunda*. In the tree-based analysis, the cluster flies were all assigned to their respective species separately from each other confirming the morphological identification. These results provide DNA barcodes that contribute to the growth of reference databases and allow fast and accurate identification.

Introduction

The family Polleniidae Brauer and Bergenstamm, 1889 has been previously considered a subfamily of Calliphoridae containing several genera and species. Recently, the subfamily Polleniinae has been raised to the family status following phylogenetic analysis [1]. The largest genus is *Pollenia* Robineau-Desvoidy, 1830 whose species are commonly called cluster flies due to their observed habit of aggregating in crevices and corners of dark parts in buildings [2]. The presence of dull colouration and yellow crinkly hairs on the head and thorax of cluster flies differentiates them from blow flies [3–5]. The adults are common pollinators [6] and can be among the most abundant flower-visiting insects in agricultural and urban areas [3].

Cluster fly larvae are internal parasites or predators of earthworms (Lumbricidae) [2,7,8]. Yet, the biology of this group is relatively poorly known [1] and a few have been observed parasitising other hosts (caterpillars and bees) [3]. *Pollenia rudis* Fabricius, 1794, the most common species in the genus, is largely distributed in the Palaearctic, Nearctic and Oriental regions [3–5]. Adult *P. rudis* are mostly herbivores and feed on sap, flowers, fruit, faeces and many different types of decaying organic matter [9]. They are mostly active during the spring and lay their eggs on the soil. After hatching, larvae actively seek earthworm hosts [3]. *Pollenia vagabunda* (Meigen, 1826) is widespread in Europe [3]. Rognes [2] has recorded *P. vagabunda* from North Morocco; however, he reported its occurrence in Algeria to be uncertain based on a dubious record. Little is known about the biology of *P. vagabunda*. This species may overwinter as adults inside buildings. The puparia of *P. vagabunda* have been found in the stems of corn infested by the moth *Sesamia nonagrioides* (Lefebvre, 1827). It was therefore suggested that the larvae may parasitise organisms other than lumbricids [2].

Urban entomology is a subfield of forensic entomology which deals with arthropod pests that harm humans and their immediate environment. The species *P. rudis* has been closely observed due to its

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tendency to infest buildings and thus its potential as a disease vector. Cluster flies tend to retire indoors in large numbers during the autumn to overwinter. They enter through cracks and holes in houses and other structures [10]. Infestations are often observed in buildings that exist at higher altitudes, as well as in buildings which are older [11]. After entering a building, cluster flies overwinter habitually in isolated areas between the walls and in the ceilings until they emerge in the spring and seek access to the outside [10]. Massive and frequent infestations over the years can damage attics, walls, or windows with fly faeces or bodies, potentially causing allergic reactions, as well as secondary infestations of dermestid beetles that breed on the dead flies [3,11]. Furthermore, *Pollenia* species can also create a fire hazard when using electric fly killers since dead insects may pile up and eventually touch the killing grid [11].

Cluster flies' role in disease transmission as mechanical vectors has been reported in numerous cases. An investigation of mesophilic bacteria carried on *P. rudis* was conducted after a large infestation of cluster flies in a German hospital in 1973. Results showed that *P. rudis* was capable of spreading bacteria causing opportunistic and/or nosocomial infections in humans. Consequently, *Pollenia* species may transmit bacterial pathogens by infesting massively delicate areas such as hospitals [12]. It has also been reported that an entire city's water reservoir tank, which was infested with cluster flies, was drained in New Zealand in 2002 due to high levels of faecal coliforms [10].

The main application of medicolegal forensic entomology is to estimate the minimum time elapsed since death, or postmortem interval (PMI). It can also be concluded that a corpse has been moved from its original location when an unexpected species is identified, which is more characteristic of a different habitat or geographic region. However, this application depends on the knowledge of the local fauna [13]. Therefore, identifying present insect species is an initial crucial step in death investigations [14]. This step can be problematic when entomologists are confronted with damaged specimens or when partial arthropod remains are the only evidence available [15]. Furthermore, it is challenging to identify intact specimens of immature stages based on morphological criteria due to the presence of similar characteristics shared between different species [14] and the lack of identification keys. Live immature specimens are usually reared to adulthood so that distinguishable features become visible for species identification [16].

Adults of *Pollenia* are not included in forensically important entomofauna because their larvae are parasites [17]. Nonetheless, they have been found on dead bodies [13], as well as on meat, fish [18], liver [18,19], and squid-baited traps in the past [20,21]. The use of squid as bait has shown promise in decomposition studies, as it retains moisture for much longer than other types of bait [20]. Adult *Pollenia* can be identified morphologically; however, larvae are mostly undescribed [11]. Some *Pollenia* species are difficult to identify based on female samples, which are more commonly encountered than males [21]. They have been indistinguishable in many studies based on morphological characteristics and misidentified as *P. rudis* as a result [3,5]. Molecular techniques have been developed to overcome such challenges, and allow identification regardless of variables such as life stage, sex, and colour morph [22]. DNA barcoding in animal species uses short strands of DNA sequences which are frequently isolated from the mitochondrial cytochrome c oxidase I gene (*COI*), as it is widely considered the standard gene for such analyses. These sequences are compared to those in various genetic databases, such as Barcode of Life Data Systems (BOLD) or GenBank (the National Center for Biotechnology Information — NCBI), using an alignment programme known as Basic Local Alignment Search Tool (BLAST) [23]. The success of molecular identification depends on the selection of a suitable marker gene and the availability of reference sequences in the databases. Therefore, this study aims to update the distribution records of Polleniidae and test the efficacy of DNA barcoding and the existing databases to identify *P. rudis* and *P. vagabunda*. This is the first study in Algeria reporting the occurrence of cluster flies on carcasses.

Material and methods

Samples

Adult flies were collected during spring (between 21 April and 8 May) of 2016 on 18 exposed male rabbit carcasses weighing approximately (2.50 ± 0.42) kg in a semi-urban site in Blida, Algeria (coordinates: 36° 30′ 38.5″ N, 2° 52′ 21.4″ E) at 188 m above sea level using a trap described in Taleb et al. [24]. The carcass and the trap were protected by a metal cage measuring 80 cm × 60 cm × 150 cm. This prevented disturbance by large vertebrate scavengers but permitted arthropod colonization. Carcasses were separated by a minimum distance of 50 m. Sampling was done at 8:00 am and 5:00 pm daily over the course of 2 weeks.

Freezing at −20 °C for 1 h is the recommended method for killing adult insects [16]; however, this method is not possible in the field. It has been shown that DNA barcoding was successful using specimens killed by ethyl acetate vapours [25–28]. Therefore, the flies were killed by exposing them to

ethyl acetate vapours for a maximum of 10 min to make sure that the DNA was not damaged. Each specimen was morphologically identified and confirmed multiple times by at least three experts using the keys of Rognes [2,5] and Jewiss-Gaines et al. [3], with Carl Zeiss® Stemi 2000-C and Leica® EZ4 HD stereo zoom microscopes coupled with digital cameras. The samples were then stored in 70%–95% ethanol and kept at −20 °C.

DNA extraction

The samples were rinsed with deionised water and dried. DNA was extracted from two legs using the DNeasy® Blood and Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocol with over-night incubation. The remaining tissue was retained as a voucher specimen and stored in 95% ethanol. Extracts were kept at 4 °C for future molecular analysis.

Polymerase chain reaction (PCR)

A region of the mitochondrial gene *COI* was amplified using primers C1–N–2191 (5′-CCCGGTAAAATT AAAATATAAACTTC-3′) and C1-J-1718 (50-GGA GGATTTGGAAATTGATTAGTTCC-3′0) [29]. PCR reactions were carried out in a final volume of 20 μL. Each PCR reaction contained 1× buffer (with 20 mmol/L Mg^{2+}), 2.5 mmol/L of each dNTP, 2.5 U AmpONE^{TM} Taq DNA polymerase (all GeneAll®, Seoul, Korea), 10 mmol/L of each primer, and 5 μL of the DNA template. The PCR was carried out in a ProFlex^{TM} 3 × 32-well PCR System thermal cycler (ThermoFisher Scientific^{TM}, Foster City, CA, USA) under the following conditions: 95 °C for 5 min, 35 cycles of 95 °C for 35 s, 54 °C for 35 s and 72 °C for 40 s. The sizes of the amplified fragments were verified using gel electrophoresis in a 1% agarose gel stained with GelRed (Biotium, Fremont, CA, USA). PCR products were purified using the commercial kit AMPure XP (Beckman Coulter, Miami, FL, USA) according to the manufacturer's instructions.

Sequencing

Sequencing was carried out using the BigDye® Terminator v3.1 sequencing kit (ThermoFisher Science^{TM}). The 15 μL reaction mix consisted of 0.5 μL BigDye® Terminator v3.1 Ready Reaction Mix (×2.5), 2.5 μL sequencing buffer BigDye (×5), 0.5 μL primer (3.2 pmol), and 2 μL PCR product. The reactions were performed using a Bio-RAD T100 thermocycler under the following conditions: 96 °C for 1 min, 25 cycles of 96 °C for 10 s, 50 °C for 25 s and 60 °C for 4 min.

Sequencing products were purified using the purification kit Clean-up Kit^{TM} (Zymo Research Corp., Irvine, CA, USA) according to the manufacturer's protocol. Automated sequencing was carried out using ABI 3130 capillary sequencer (Applied Biosystem, Foster City, CA, USA).

Genetic analysis

Raw sequences were quality trimmed and manually aligned using BioEdit v7.2.5 [30]. The obtained sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/) and BOLD (http://www.boldsystems.org/). We additionally retrieved publicly available *COI* sequences referring to *Pollenia* from the BOLD and GenBank databases to include in the genetic analysis (Table 1). The sequences were aligned using MUSCLE [31].

Genetic distances in barcoding studies are usually determined using the Kimura's Two-Parameter (K2P) model [32] following Hebert et al. [33]. However, it was shown that the use of K2P for closely related *COI* sequences was inappropriate and unnecessary and that uncorrected *p*-distances should be used instead [34–36]. Moreover, our preliminary analysis has not revealed any significant difference between the two methods. Thus, uncorrected *p*-distances were calculated in MEGA7 [37] to study *COI* intra- and interspecific divergence. The phylogenetic tree was constructed with the maximum parsimony method (MP) using PAUP*4.0 software and 10 000 bootstrap replicates [38]. The *COI* sequence of the blow fly *Calliphora alaskensis* (Shannon, 1923) (GenBank accession number JN263392) was used as the outgroup in the phylogenetic analysis.

Results and discussion

Occurrence on cadavers

Pollenia species have never been reported from decomposition studies in Algeria. Thus, the current data present the first approach of using molecular tools to identify cluster flies from Algeria (in the context of a decomposition study). A total number of 157 adults of *P. rudis* were collected together with 325 adults of *P. vagabunda* (Table 2). *Pollenia* species are usually neglected during carrion decomposition studies and are often identified to the genus level. Specimens of *Pollenia*, particularly those of *P. rudis*, have frequently been reported from carrion succession experiments in Europe and North America [17,18,39–41]. *Pollenia* male samples were completely absent in the collections of the current study. Similarly, Martín-Vega and Baz [21] did not collect any *Pollenia* male individuals on traps baited with squid. In contrast, Baz et al. [20] reported both males and females of *Pollenia* species with female-biased sex ratio, also *via* the use of squid-baited traps.

Table 1. List of species used for genetic analysis with their GenBank accession numbers and/or BOLD sequence ID.

Species	GenBank accession No.	BOLD sequence ID	Country
Pollenia amentaria (Scopoli, 1763)	GQ409350.1	–	Unknown
	–	GMGMG197-14	Germany
Pollenia angustigena Wainwright, 1940	KY749783	GBMIN53895-17	Poland
Pollenia griseotomentosa (Jacentkovský, 1944)	JF867619	BBDCN031-10	Canada
	–	GMGMB1441-14	Germany
	KR743688	SMTPB3144-13	Canada
Pollenia hungarica Rognes, 1987	–	GMGMH527-14	Germany
	MK883262	–	China
Pollenia labialis Robineau-Desvoidy, 1863	MG755746	–	China
	HM883114	BBDED147-10	Canada
	JQ807043.1	–	USA
Pollenia leclercqiana Lehrer, 1978	KY860000	–	Portugal
Pollenia pediculata Macquart, 1834	MG118651	AGAKR005-17	Canada
	–	GMEGA657-14	Egypt
	–	GBMWB1680-15	Germany
	KT368668	GBMIN53897-17	Ireland
	MG673561	GBDP30823-19	Norway
	KY749784	GBMIN53899-17	Poland
	JQ807044	–	USA
Pollenia rudis Fabricius, 1794	MT299918	DZPO001-20	Algeria
	MT299919	DZPO002-20	Algeria
	MT299920	DZPO003-20	Algeria
	MT299921	DZPO004-20	Algeria
	MT299922	DZPO005-20	Algeria
	MT299923	DZPO006-20	Algeria
	FR719179	GBMIN30477-13	UK
	JQ807056	–	USA
	MG121409	ASDMT856-11	Canada
	KT368666	GBMIN53901-17	Ireland
	KY749785	GBDP30488-19	Poland
	–	GBMWB1072-15	Germany
Pollenia vagabunda (Meigen, 1826)	MT309586	DZPO007-20	Algeria
	MT309587	DZPO008-20	Algeria
	MT309588	DZPO009-20	Algeria
	MT309589	DZPO010-20	Algeria
	MT309590	DZPO011-20	Algeria
	MT309592	DZPO012-20	Algeria
	–	GMGMG088-14	Germany
	MK883263	–	China
	MG120403	ASDMT1354-11	Canada

Table 2. Absolute abundance of adult *Pollenia rudis* and *Pollenia vagabunda* according to the decomposition stages of rabbit carcasses.

Species	Decomposition stage				
	Fresh	Bloated	Decay	Dry	Total
Pollenia rudis	0	0	30	127	157
Pollenia vagabunda	0	42	47	236	325

Occurrence according to stages of decomposition

Stages of decomposition were defined according to the classification of Reed [42], which establishes four stages; in order of increasing decomposition time, these are named the fresh stage, bloated stage, decay stage, and dry stage. This classification was used in our study because it is more practical to study the decomposition of small carrion. In the current study, *P. rudis* was recorded in the decay and dry stages whereas *P. vagabunda* was collected in the bloated stage and continued to appear until the dry stage. Prado e Castro [40] noted the presence of *Pollenia* spp. in Portugal on pig carcasses during spring in all the stages of decomposition and during summer from the bloated until the dry stage.

Tabor [41] reported the occurrence of *P. rudis* in the USA on pig carcasses during spring in the bloated stage and during summer in every stage of decomposition. Consequently, *Pollenia* species do not seem to be attracted to a specific stage of decomposition. Decomposing remains are attractive to *Pollenia*, yet their significance in medicolegal entomology is neglected due to the absence of correlation to a particular stage of decomposition [18].

Temporal occurrence

In the present study, the occurrence of *P. rudis* and *P. vagabunda* in spring is consistent with the observations reported by other authors. *Pollenia* spp. were found to be abundant particularly in spring followed by summer [40,41]. Szpila [43] stated that *P. rudis* occurred in Poland mainly during spring and summer in very high abundance in grassland. Adult *P. vagabunda* were recorded in Poland during summer in low numbers [43]. It was found that *P. rudis* populations in Canada peaked in April and June [3].

Ecological role

The presence of adult *Pollenia* in high numbers, as observed during this study as well as others, shows that their occurrence on cadavers is unlikely to be accidental. Šuláková and Barták [18] suggested that these species could be attracted to the liquid pouring out of the baits. Arthropod species found on decomposing remains are attributed to ecological categories. These are generally classified into four classes, namely necrophagous species, parasites and predators on the necrophagous species, omnivorous species, and adventive species [44]. A fifth category is sometimes cited; these are the so-called accidental species whose presence on the body is the result of chance [45]. Šuláková and Barták [18] proposed to attribute to *Pollenia* the status of "predator/parasite on necrophagous species", following the classification proposed by Smith [44] since earthworms and snails have frequently been observed participating in decomposition in experiments and real cases. Nonetheless, the occurrence of *Pollenia* spp. on carrion seems to be related to the feeding of the adult flies on cadavers as females feed on liquid and semi-liquid decomposition products. However, the larvae of these true parasites cannot finish their development by feeding on cadavers [43].

Molecular identification

The DNeasy® Blood and Tissue kit (Qiagen) yielded adequate DNA for *COI* amplification of all the specimens tested. DNA concentrations varied between 4 ng/μL and 8 ng/μL with small variation in the average DNA yield between *P. rudis* (6.05 ± 1.93 ng/μL) and *P. vagabunda* (6.13 ± 1.67 ng/μL). Ames et al. [46] reported DNA concentrations of *Calliphora vicina* Robineau-Desvoidy, 1830 and *Calliphora vomitoria* (Linnaeus, 1758) ranging from 0.2 ng/mL from a single wing to 30 ng/mL for a complete adult fly. These authors found little variation in the DNA yield between these two species which is comparable to our result. Data from the literature as well as our results have shown that using minimal fly tissue leads to successful DNA extraction and amplification [47]. This can be useful when only

insect parts (legs, wings, etc.) are found at crime scenes. Additionally, it is always helpful to keep the sample for the possibility of a morphological re-examination, if necessary. Fragments of the *COI* gene of *P. rudis* and *P. vagabunda* were amplified and sequenced successfully. The PCR amplification produced fragments of approximately 500 bp.

All the sequences were correctly identified using the BLASTn tool from the GenBank database and the BOLD identification engine. Meiklejohn et al. [23] considered molecular identification reliable when multiple independent records with the same top match statistics had the correct taxonomic name which is in agreement with our findings. The GenBank percent identity of the first match was (99.16 ± 0.13)%, whereas the BOLD percent of similarity of the first match was (99.43 ± 0.09)%.

Genetic variation

The intra- and interspecific divergence is shown in Table 3. No intraspecific variation was observed within *P. rudis* sequences while the divergence between *P. vagabunda* sequences varied from 0 to 0.33%. No intraspecific divergence amongst the samples of *P. rudis* and *P. vagabunda* from different countries was found. As for the genetic variation between *P. rudis* and *P. vagabunda* specimens sequenced in this study, an average value of 9.59% was found. Many studies have attempted to determine the standard threshold for intra- and interspecific divergences. For instance, Hebert et al. [48] reported a rule for the intraspecific variation of being <3% and interspecific divergence at least 10 times greater than the intraspecific divergence. Wells and Sperling [49] who studied the genetic variation of necrophagous fly species reported the percentage of intraspecific divergence being less than or equal to 1% for the sequences of cytochrome c oxidase I and II (*COI* + *COII*) and the percentage of intraspecific divergence being greater than 3% as adequate thresholds. In general, intraspecific variations are commonly less than 1% and rarely greater than 2% [50]. Intra- and interspecific divergence values of our sequences were

Table 3. Intra- and interspecific *P*-distances of the analysed cytochrome c oxidase I gene (*COI*) expressed as percentages.

No.	Species	Intra.	1	2	3	4	5	6	7	8	9	10	11
1	*P. rudis* DZ	0											
2	*P. vagabunda* DZ	0–0.33	9.59										
3	*P. rudis* W	0	0.66	10.25									
4	*P. vagabunda* W	0	9.87	0.71	9.87								
5	*P. pediculata* W	0–0.66	3.10	8.42	3.76	8.69							
6	*P. amentaria* W	0.66	8.55	8.61	9.21	8.88	8.04						
7	*P. hungarica* W	0.30	3.78	8.94	4.44	9.21	2.94	9.87					
8	*P. leclercqiana* PT	–	8.22	10.91	8.88	11.18	8.18	7.89	9.87				
9	*P. labialis* W	0–0.33	8.22	8.50	8.22	8.44	8.36	9.10	8.55	9.43			
10	*P. griseotomentosa* W	0	9.21	8.94	9.87	9.21	8.36	8.55	8.55	9.87	7.79		
11	*P. angustigena* W	0.33	5.43	8.44	6.09	8.72	7.18	9.38	5.76	8.72	7.62	6.74	
12	*P. alaskensis*	–	9.54	11.24	10.20	11.84	9.35	7.24	10.69	10.20	9.87	9.87	11.02

DZ: Algeria; Intra.: intraspecific divergence; PT: Portugal; W: world.

found in agreement with the permissible limits, i.e. <1% and >3%, respectively [48,49]. *COI* barcodes obtained from this study were robust enough to identify and distinguish unambiguously between *P. rudis* and *P. vagabunda*. Nonetheless, these rules cannot be generalised to all groups as *COI* barcoding may fail to discriminate between sister species [51].

The divergence between *P. rudis* sequences from Algeria to the ones from different parts of the world was higher (0.66%). Likewise, the Algerian sequences of *P. vagabunda* showed greater divergence to those of different countries (0.71%). These findings may be a result of a gene pool fragmentation caused by geographical isolation and genetic disparity [48]. Furthermore, silent substitutions not affecting the amino-acid sequence have been reported as the source of haplotype diversity amongst fly species of forensic importance [52].

Phylogenetic analysis

Based on our preliminary analysis of the submitted *COI* sequences, the most reliable sequences within the genus *Pollenia* were chosen for the phylogenetic analysis in order to verify our species identification accuracy. Some of the *P. rudis* sequences from Spain, Portugal, and Norway (GenBank accession numbers KX161505, JX438050, and MG673730, respectively) may have been wrongly identified. For this reason, they were excluded from the analysis.

In the tree-based analysis, the Algerian cluster flies were all assigned to their respective species. At the species level, the nine *Pollenia* species formed monophyletic clusters with very robust supportive values (Figure 1). Thus, *P. rudis* and *P. vagabunda* individuals from Algeria clustered separately from each other confirming the morphological identification.
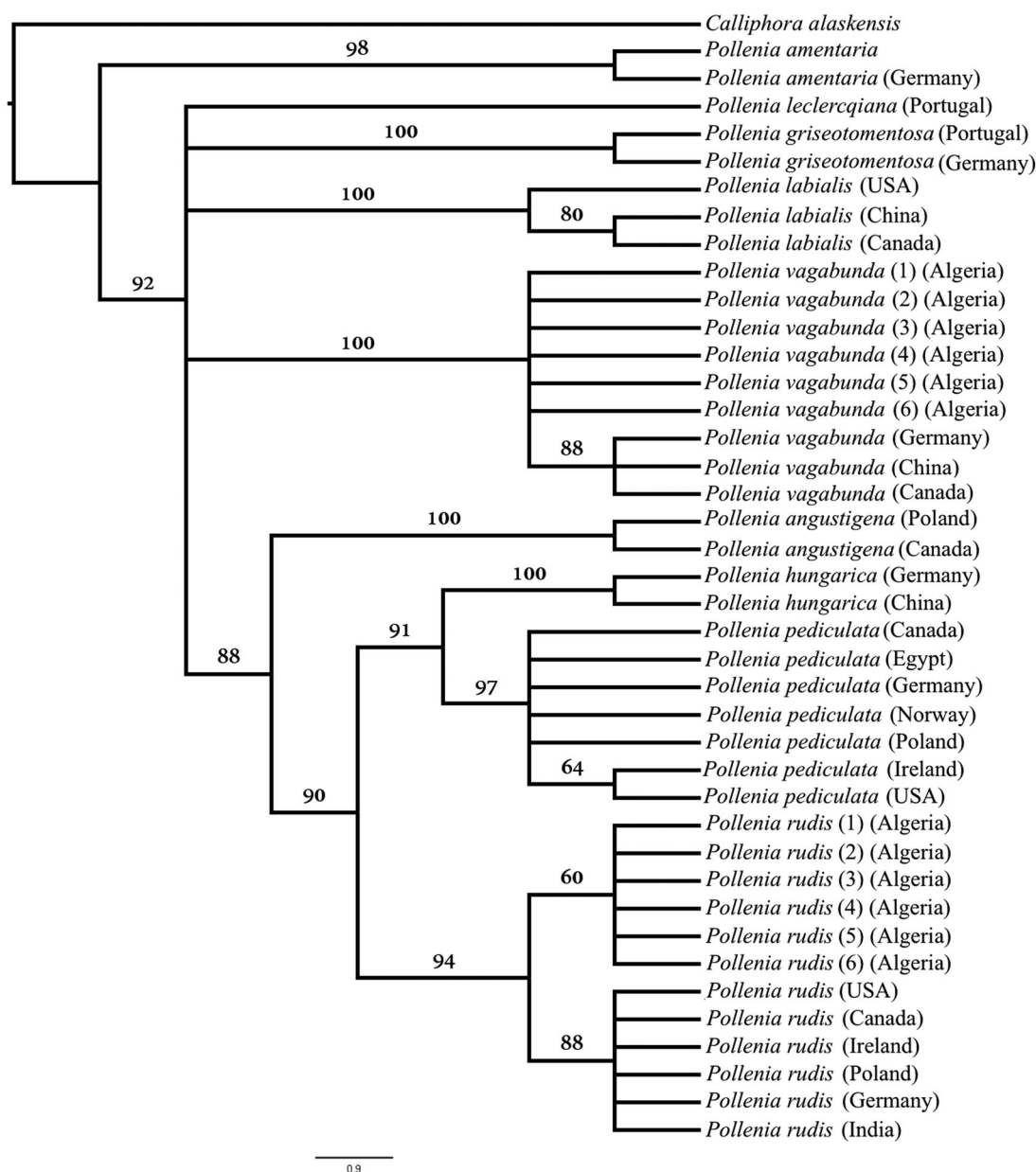

Figure 1. Maximum parsimony consensus phylogram of 41 cytochrome c oxidase I (*COI*) sequences from nine *Pollenia* species and one outgroup (*Calliphora alaskensis*) based on 10 000 bootstrap replicates.

This shows that the *COI* marker was sufficient to differentiate between *P. rudis* and *P. vagabunda*.

The phylogenetic analysis of *Pollenia COI* sequences was congruent with the monophyletic species-groups described by Rognes [2,5,7,8]. Rognes [5] divided the *P. rudis* species-group into seven distinct species: *P. angustigena* Wainwright, 1940, *P. hungarica* Rognes, 1987, *P. paupera* Rondani, 1862 (*P. longitheca* Rognes, 1987), *P. luteovillosa* Rognes, 1987, *P. pediculata* Macquart, 1834 (*P. pseudorudis* Rognes, 1985), *P. rudis* and *Pollenia* sp. Rognes [2] described five species in the *vagabunda* species-group: *P. bezziana* Rognes, 1992, *P. stigi* Rognes, 1992, *P. verneri* Rognes, 1992, *P. contempta* Robineau-Desvoidy, 1863 and *P. vagabunda*. Nevertheless, only *P. vagabunda COI* sequences are available online. Sequences of *P. angustigena*, *P. hungarica*, *P. pediculata*, and *P. rudis* formed a unique clade. Therefore, the present study supports the monophyly of the *rudis* species-group. Our study has also showed *P. hungarica* forming a sister clade with *P. pediculata* (bootstrap value of 91%). *P. rudis* has formed two separate subclades within its clade showing the Algerian *P. rudis* as a separate group from the rest of the world. Stevens et al. [53] have also reported the presence of intraspecific divergence and geographical disparities between blow fly (Calliphoridae) populations. However, it is important to use multiple genetic markers and a large number of specimens to thoroughly examine the population assembly and the accurate genetic divergence within a species [28].

Conclusion

The present study was the first molecular approach to identify cluster flies from Algeria and record their occurrence on carcasses. *Pollenia* species are found on carrion particularly in spring without being restricted to a particular stage of decomposition. Although they are not considered primary species in cadaver analysis, cluster flies should be included in carrion entomofauna. *COI* barcodes obtained from this study were robust enough to identify and distinguish unambiguously between *P. rudis* and *P. vagabunda*. Taxonomic identification through DNA barcoding should be supported by accurate morphological identification and proper genetic analyses to submit reliable DNA sequences to reference databases. Our results provide DNA barcodes that contribute to the growth of reference databases and allow fast and accurate identification. More studies are required to increase the availability of DNA barcodes and new molecular markers should be tested.

Authors' contributions

Meriem Taleb and Halide Nihal Açıkgöz designed the study and did the morphological identification and the molecular analyses. Ghania Tail supervised the study and edited the manuscript. Meriem Taleb gathered the fly samples, analysed the data and wrote the manuscript. All the authors read and approved the final manuscript.

Compliance with ethical standard

This study was approved by the Institutional Review Board of University of Blida 1 (USDB1-SNV).

Disclosure statement

No potential conflict of interest was reported by the authors.

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