Genetics & Biodiversity Journal

Journal homepage: https://journals.univ tlemcen.dz/GABJ/index.php/GABJ

ISSN: 2588-185X

Original Research Paper

Phylogenetic study of the genus *Miniopterus*.

Derouiche L[1]*, Aissi A[2], Bouali K[2]

[1]*Higher School of Food Sciences & Agri-food Industries Algiers, Algeria.*
[2]*Nature and Life Sciences Faculty, Saad Dahlab University of Blida (USDB 1), Blida, Algeria.*

***Corresponding Author**: Derouiche Louiza, Higher School of Food Sciences & Agri-food Industries Algiers, Algeria; **E-mail:** derouiche@essaia.dz

Article history: Received: June 4, 2024; Revised: July 4, 2024; Accepted: July 9, 2024

Abstract

With over 1400 species worldwide, the genus *Miniopterus* (Bonaparte, 1837) is among the most widespread genera globally. However, the phylogenetic relationships within this genus are poorly understood due to their morphological uniformity, complicating classification and species delimitation. This study addresses these challenges by employing various bioinformatics tools to analyze mitochondrial DNA sequences of the 'Cytochrome b' gene sourced from GenBank. Phylogenetic tree reconstruction was performed using MEGA, haplotype identification was conducted with DnaSP, and phylogenetic networks were analyzed using NetWork. Our findings confirm the monophyly of *Miniopterus* in two distinct geographic regions: Afro-European and Australasian-Asian. Furthermore, morphological identification in Algeria reveals the presence of only one species, *Miniopterus schreibersii*.

Keywords: *Miniopterus*, GenBank, Cytochrome b, bioinformatics, phylogenetic.

الخلاصة

مع وجود أكثر من 1400 نوع في جميع أنحاء العالم، يعد جنس المنيوبتروس (بونابرت، 1837) من بين الأجناس الأكثر انتشارًا على مستوى العالم. أشارت دراسات مختلفة إلى أن العلاقات التطورية بين الأنواع من هذا الجنس لا تزال غير مفهومة بشكل جيد بسبب التوحيد المورفولوجي الواضح الذي يقدمه الجنس، مما يعيق الإجماع على تصنيفها وحدود الأنواع. ولمعالجة هذا الأمر، أجرينا هذه الدراسة التطورية باستخدام أدوات المعلوماتية الحيوية المختلفة. يتضمن البرنامج المستخدم برنامج لإعادة بناء شجرة النشوء والتطور، وبرنامج لتحديد النمط الفرداني، وبرنامج ثالث لبناء شبكات النشوء والتطور. تم تطبيق هذه التحليلات على مورثة من تسلسل الحمض النووي للميتوكوندريا التي تم الحصول عليها من بنك المعلومات الوراثية لمختلف الأنواع والأنواع الفرعية من مناطق جغرافية مختلفة. توضح النتائج توزع جنس المنيوبتروس في منطقتين جغرافيتين متميزتين، إحداهما أفريقية أوروبية والأخرى أسترالية آسيوية. بالإضافة إلى ذلك، يشير التحديد المورفولوجي في الجزائر إلى وجود نوع واحد فقط

الكلمات الرئيسية: المنيوبتروس ، مورثة ، المعلوماتية الحيوية ، العلاقات التطورية.

Introduction

The order Chiroptera represents a unique group of ancient mammals that first appeared on Earth approximately 65 million years ago (Teeling et *al*., 2005). Members of this order have developed autonomous flight as their primary mode of locomotion (Meganathan et *al*., 2011). Chiroptera is particularly notable for the diversity in their morphology, habits, and habitats, encompassing around 1400 species worldwide (Stevens et *al*., 2006), which are divided into 175 genera within 20 families (Joffrin, 2019). Among these, seven families are particularly well-known: Rhinolophidae (Gray, 1825), Vespertilionidae (Gray, 1821), Molossidae (Gervais, 1856), Emballonuridae (Gervais, 1856), Rhinopomatidae (Bonaparte, 1838), Hipposideridae (Lydekker, 1891), and Miniopteridae (Bonaparte, 1837).

Although the family Miniopteridae is represented by a single genus, *Miniopterus* (Goodman et *al*., 2011), it is one of the most widespread mammalian families globally (Miller-Butterworth et *al*., 2007). The genus *Miniopterus*, often referred to as long-toed or curved-winged bats, comprises up to 35 species (Demos, 2019).

Phylogeny, an area of research essential for understanding different species, whether animal or plant, helps classify species based on their evolutionary relationships (Patwardhan et *al*., 2014). Modern phylogenies are typically represented by phylogenetic trees, which illustrate hypotheses of species' vertical evolution (Lord, 2015). Concurrently, bioinformatics provides central, globally accessible databases that enable scientists to present, search, and analyze information. It offers software for data analysis and comparison, and tools for modeling, visualization, exploitation, and interpretation of data (Yvan et *al*., 2007). Using various bioinformatics software, we conducted a phylogenetic study of the genus *Miniopterus*, one of the most widespread bat genera but with several uncertainties in its classification due to apparent morphological uniformity. Our objective is to reconstruct the phylogenetic tree characterizing the specific evolution of the *Miniopterus* genus and to compare the different species within it.

Material and methods

Our study utilized already published sequences obtained through Polymerase Chain Reaction (PCR) (Kleppe et *al*., 1971) and sanger sequencing. The researchers who published the results collected the samples, extracted the DNA, amplified a targeted DNA fragment, and sequenced that fragment. These sequences, generated by various research laboratories globally, were then submitted to biological data repositories such as GenBank, ensuring the data is freely accessible to researchers worldwide (Sayers et *al*., 2020).

We extracted all available DNA sequences of the *Miniopterus* genus from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Subsequent analysis and classification of these sequences were performed using multiple molecular data processing software tools.

- FaBox (Toolbox for Fasta Sequences): A collection of simple, intuitive web services designed for quick tasks involving sequence data, enabling biologists and medical researchers to efficiently process and analyze sequences (Villesen, 2007).
- MEGA (Molecular Evolutionary Genetics Analysis): This software provides integrated tools for statistical analysis of DNA and protein sequence data from an evolutionary perspective. It constructs sequence alignments and phylogenetic trees, allowing the application of evolutionary bioinformatics methods in biology, biomedicine, and evolution (Tamura et *al*., 2011; Yoann, 2012).
- DnaSP (DNA Sequence Polymorphism Analysis of Large Data Sets): A bioinformatics tool designed for comprehensive analysis of DNA sequence variation, facilitating detailed polymorphism studies (Rozas et *al*., 2017).
- NetWork Software: Utilized for reconstructing phylogenetic networks, this software infers ancestral types, potential evolutionary paths, branching patterns, and estimates datings. It generates evolutionary trees and networks from genetic, linguistic, and other data (Huson and Bryant, 2012; Forster and Forster, 2020).

Results and discussion

GenBank result

Our analysis of 51.717 DNA sequences of the *Miniopterus* genus from GenBank enabled us to inventory all published species and classify the genes utilized for each. The Cytochrome b (Cytb) gene emerged as the most frequently used gene for studying the majority of species. The detailed results are presented in table I.

The Cytb gene, a mitochondrial gene involved in electron transport in the mitochondrial chain, is extensively used in systematic studies to resolve taxonomic discrepancies across various levels (Izeni et *al*., 2001). According to Demos (2019), mitochondrial markers are particularly effective for phylogenetic studies aimed at determining sister taxa. Consequently, our study focused on the Cytochrome b gene for the phylogenetic analysis of the *Miniopterus* genus.

Table I. DNA sequences of the genus *Miniopterus* extract from GenBank.

Species	Total number of sequences	Number of Cytb sequences
Miniopterus aelleni	33	11
Miniopterus cf. aelleni A	6	6
Miniopterus cf. aelleni B	14	14
Miniopterus africanus	16	2
Miniopterus ambohitrensis	6	1

Miniopterus australis	21	2
Miniopterus fraterculus	106	50
Miniopterus fuliginosus	15900	1
Miniopterus gleni	45	35
Miniopterus griffithsi	16	6
Miniopterus griveaudi griveaudi	10	10
Miniopterus inflatus rufus	1	1
Miniopterus macroceneme	5	1
Miniopterus maghrebensis	14	14
Miniopterus magnater	67	7
Miniopterus mahafaliensis	28	18
Miniopterus majori	55	44
Miniopterus manavi	33	11
Miniopterus medius	4	1
Miniopterus minor	57	7
Miniopterus mossambicus	36	16
Miniopterus newtonii	4	4
Miniopterus schreibersii bassanii	11	1
Miniopterus schreibersii oceanensis	25	2
Miniopterus paululus	18	3
Miniopterus schreibersii blepotis	2	1
Miniopterus petersoni	21	10
Miniopterus schreibersii orianae	3	2
Miniopterus schreibersii pallidus	126	66
Miniopterus schreibersii schreibersii	69	59
Miniopterus schreibersii villiersi	3	3
Miniopterus sororculus	24	13
Miniopterus griveaudi	157	2
Miniopterus inflatus	32	1
Miniopterus natalensis	33529	37
Miniopterus schreibersii	928	7
Miniopterus sp. B165	1	1
Miniopterus sp. B76	1	1
Miniopterus sp. B96	1	1
Miniopterus sp. BBRA-2009c	33	23
Miniopterus *sp. BRA-2011*	25	15
Miniopterus sp. clade 1 TD-20	68	43
Miniopterus sp. clade 10 TD-2020	33	20
Miniopterus sp. clade 2 TD-2020	40	25
Miniopterus sp. clade 3 TD-2020	6	1
Miniopterus sp. clade 4 TD-2020	46	26
Miniopterus sp. clade 5 TD-2020	25	11
Miniopterus sp. clade 6 TD-2020	6	1
Miniopterus sp. clade 7 TD-2020	37	22
Miniopterus sp. clade 8 TD-2020	29	14
Miniopterus sp. clade 9 TD-2020	20	5
Miniopterus sp. Comoros clade 2	9	3
Miniopterus sp. FMNH 167450	1	1
Miniopterus sp. FMNH 172602	1	1
Miniopterus sp. n. TM-2020	5	5
Miniopterus sp. P3	1	1
Miniopterus sp. P4	5	5
Miniopterus sp. P5	3	3
Miniopterus sp. P6	8	8
Miniopterus sp. P7	1	8
Miniopterus sp. SMG-2014a	1	1
Miniopterus sp. Sororculus	5	5
Miniopterus sp. X3	1	1

The phylogenetic trees obtained

The construction of the phylogenetic tree was performed using the MEGA software. We selected representative Cytb sequences based on their geographical origin and nucleotide base pair (bp) length. Notably, there was significant size variability among Cytb gene sequences, complicating alignment. To address this, we separately processed short sequences (between 222bp and 833bp) and determined their positions relative to long sequences (between 1010bp and 1140bp). This approach allowed us to categorize the sequences into two groups, A and B (Fig. 1).

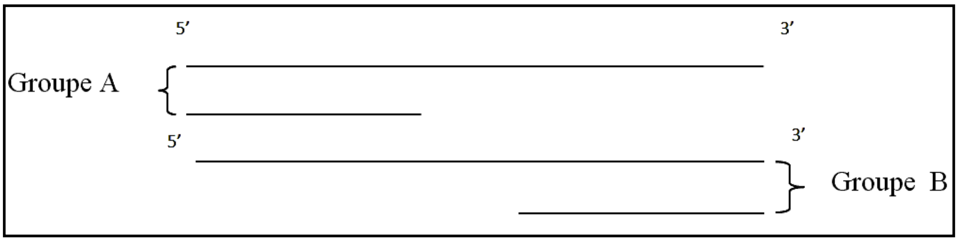

Fig. 1. Diagram explaining the positions of small versus large sequences in the alignment.

1. Phylogenetic tree of Cytb group A

Group A includes all the long sequences aligned with the short sequences which are at the 5' position. The relationships between the Cytb sequences have been represented in the following tree in figure 2.

Upon analyzing the phylogenetic tree derived from group A of the genus *Miniopterus*, we identified two monophyletic clades distributed across distinct geographical regions:

- **Clade 1 Australasia-Asia:** Includes eight taxa from Australasia and Asia: *Miniopterus pusillus, Miniopterus medius, Miniopterus australis, Miniopterus macroceneme, Miniopterus paululus, Miniopterus schreibersii, Miniopterus magnater, Miniopterus fulginosus.*

  This clade is subdivided into two subclades:

  Sub clade A which is divided into:

  A1: contains the species *Miniopterus schreibersii*, *Miniopterus magnate* and *Miniopterus fulginosus*.

  A2: contains the subspecies *Miniopterus schreibersii orianae, Miniopterus schreibersii oceanensis, Miniopterus schreibersii bassanii* and *Miniopterus schreibersii blepotis*.

  Sub clade B which is divided into:

  B1: contains the species *Miniopterus australis* and *Miniopterus macroceneme*.

  B2: represented by the species *Miniopterus pusillus, Miniopterus medius, Miniopterus australis* and *Miniopterus paululus.*

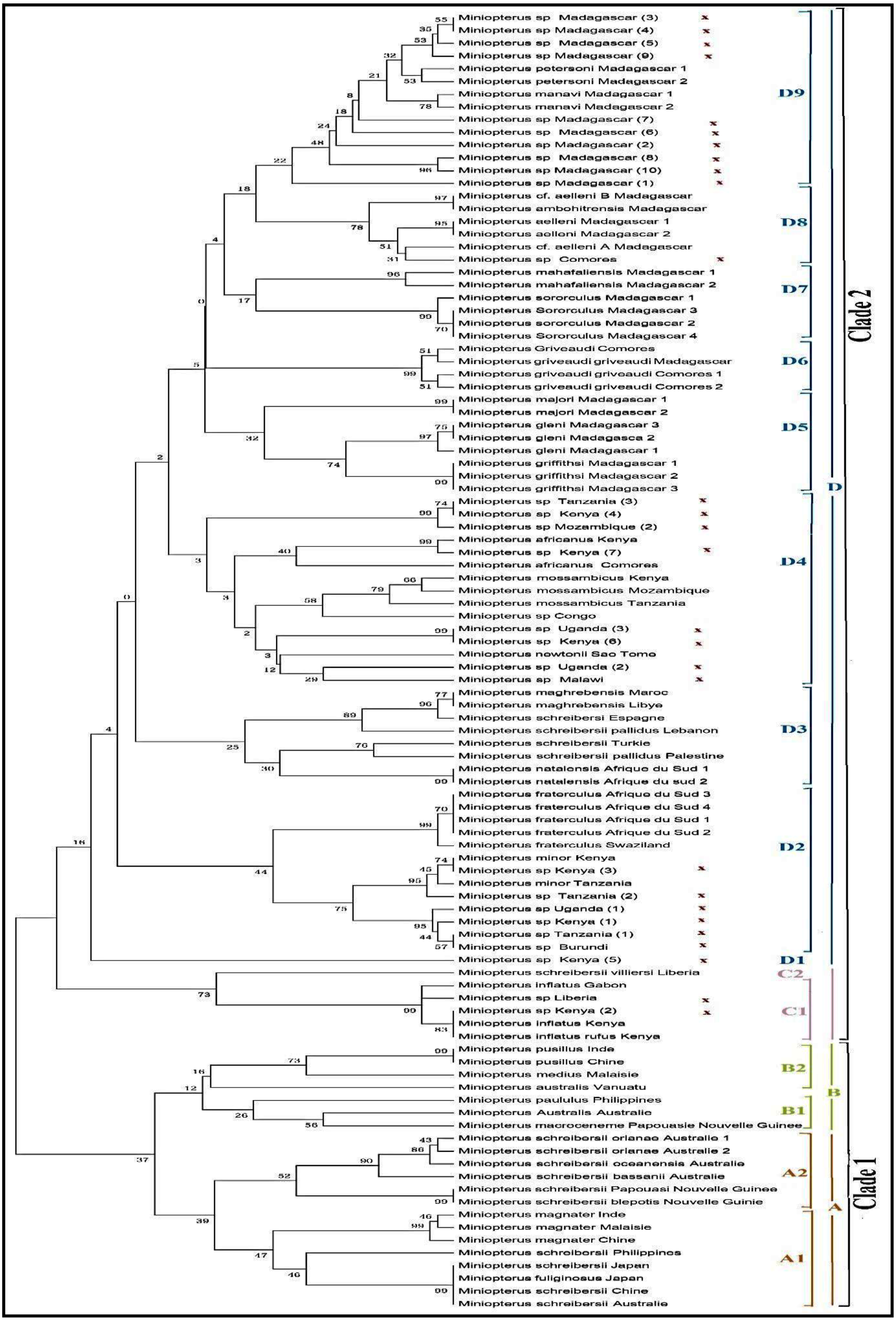

Fig. 2. Phylogenetic tree reconstructed by the UPGMA method based on Cytb sequences from group A of the *Miniopterus* genus using the MEGA software.

- **Clade 2 African-European:** brings together 22 species which are: *Miniopterus petersoni, Miniopterus manavi, Miniopterus aelleni, Miniopterus cf. aelleni A, Miniopterus cf. aelleni B, Miniopterus ambohitrensis, Miniopterus mahafaliensis, Miniopterus sororculus, Miniopterus griveaudi, Miniopterus majori, Miniopterus gleni, Miniopterus griffithsi, Miniopterus africanus, Miniopterus mossambicus, Miniopterus newtonii, Miniopterusnatalensis, Miniopterus schreibersii, Miniopterus macroceneme, Miniopterus maghrebensis, Miniopterus fra terculus, Miniopterus minor* and *Miniopterus inflatus.*

This clade is subdivided into 2 subclades:

Sub-clade C which is divided into:

C1: contains the species *Miniopterus inflatus* and the subspecies *Miniopterus inflatus rufus*.

C1: represents the subspecies *Miniopterus schreibersii villersi*.

Sub clade D which is the most branched, it is divided into:

D1: represents a single unclassified species *Miniopterus sp*.

D2: contains two species *Miniopterus fraterculus* and *Miniopterus minor*.

D3: contains the species *Miniopterus maghrebensis, Miniopterus schreibersii, Miniopterus natalensis*, with the subspecies *Miniopterus schreibersii pallidus*.

D4: includes three species *Miniopterus africanus, Miniopterus mossambicus* and *Miniopterus newtonii*.

D5: includes three species *Miniopterus gleni, Miniopterus griffithsi* and *Miniopterus majori*.

D6: represents a species *Miniopterus griveaudi* and a subspecies *Miniopterus griveaudi griveaudi*.

D7: includes *Miniopterus mahafaliensis* and *Miniopterus sororculus*.

D8: contains the *Miniopterus* species *cf. aelleni B, Miniopterus ambhitrensis, Miniopterus cf. aelleni A* and *Miniopterus aelleni.*

D9: contains *Miniopterus petersoni* and *Miniopterus manavi.*

2. Phylogenetic tree of Cytb group B

Group B comprises all the long sequences aligned with the short sequences at the 3' position. The relationships between the Cytb sequences within this group are illustrated in the phylogenetic tree shown in figure 3.

According to the tree of group B of the genus *Miniopterus*, we noticed two main clades which are divided into several subclades:

- **Clade 1 Australasia-Asia:** Brings together 5 different species which are: *Miniopterus paululus, Miniopterus medius, Miniopterus magnater, Miniopterus fuliginosus,* and *Miniopterus pusillus*.

Sub clade A: divides into:
A1: includes the species *Miniopterus magnater* and *Miniopterus fuliginosus*.

A2: contains the species *Miniopterus medius* and *Miniopterus pusillus*.

Sub clade B: represents a single species *Miniopterus paululus*.

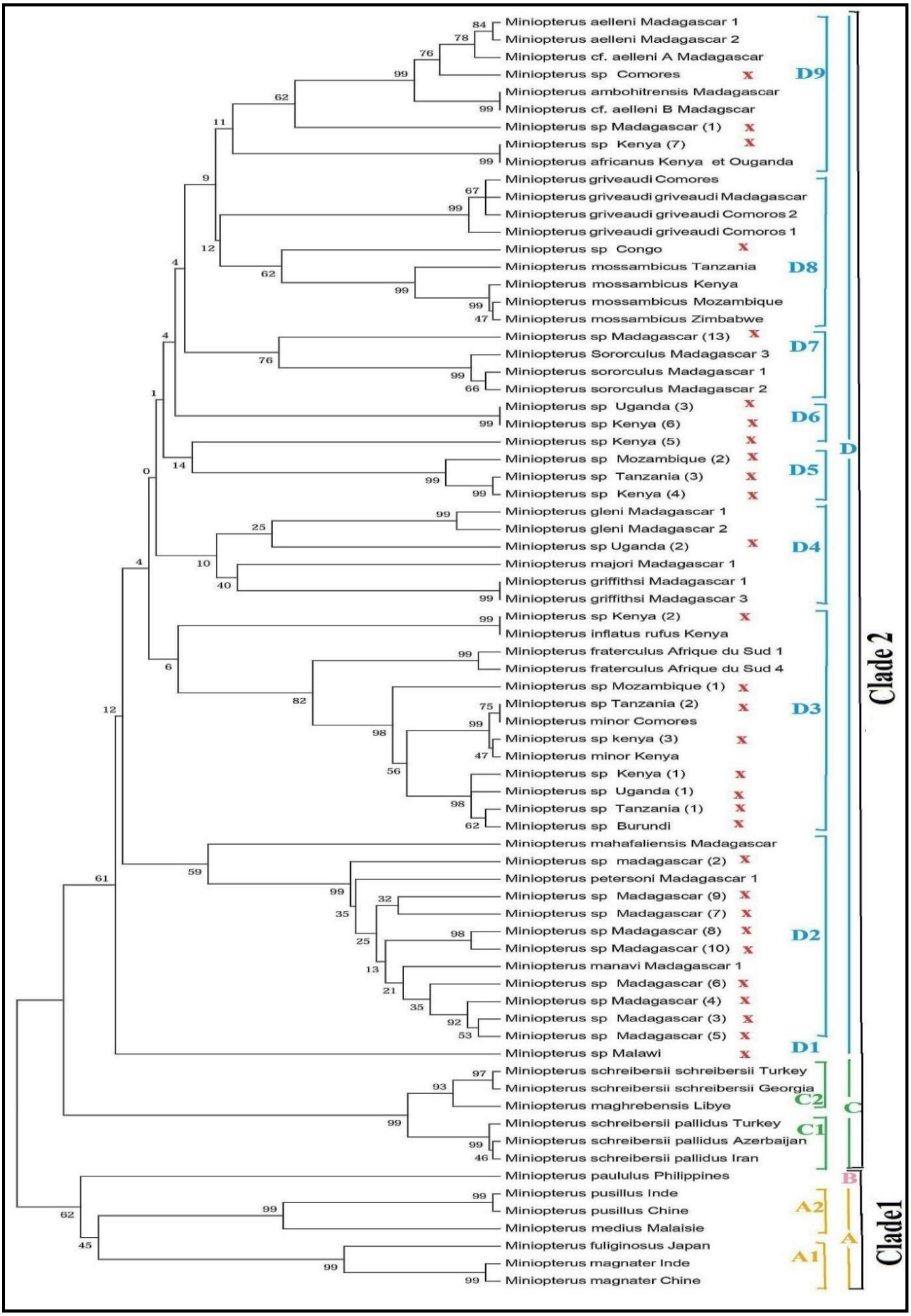

Fig. 3. Phylogenetic tree reconstructed by the UPGMA method based on Cytochrome b sequences from group B of the genus *Miniopterus* using MEGA.

- **Clade 2 African-European:** brings together 18 different species which are: *Miniopterus petersoni, Miniopterus manavi, Miniopterus aelleni, Miniopterus cf. aelleni A, Miniopterus cf. aelleni B, Miniopterus ambohitrensis, Miniopterus mahafaliensis, Miniopterus sororculus, Miniopterus griveaudi, Miniopterus majori, Miniopterus gleni, Miniopterus griffithsi, Miniopterus africanus, Miniopterus mossambicus, Miniopterus maghrebensis, Miniopterus fraterculus*, *Miniopterus minor* and *Miniopterus inflatus.*

  Sub-clade C: divides into:
  C1: contains the subspecies *Miniopterus schreibersii palludis*.
  C2: includes the species *Miniopterus maghrebensis* and the subspecies *Miniopterus schreibersii schreibersii.*
  Sub clade D: it is the most branched, it is divided into:
  D1: represented by a single subspecies *Miniopterus sp Malawi*.
  D2: contains the species *Miniopterus petersoni*, *Miniopterus manavi* and *Miniopterus mahafaliensis*.
  D3: represented by the species *Miniopterus minor, Miniopterus fraterculus* and the subspecies *Miniopterus inflatus rufus*.
  D4: includes the species *Miniopterus gleni*, *Miniopterus griffithsi* and *Miniopterus majori*.
  D5: contains three unclassified species *Miniopterus sp* Mozambique (2), *Miniopterus sp* Tanzania (3), and *Miniopterus sp* Kenya (4).
  D6: represents three unclassified species *Miniopterus sp* Uganda (3), *Miniopterus sp* Kenya (5) and *Miniopterus sp* Kenya (6).
  D7: contains a species *Miniopterus sororculus*.
  D8: represented by two species *Miniopterus griveaudi* and *Miniopterus mossambicus*, and one subspecies *Miniopterus griveaudi griveaudi*.
  D9: contains the species *Miniopterus aelleni*, *Miniopterus cf. aelleni A, Miniopterus cf. aelleni B, Miniopterus ambohitrensis* and *Miniopterus africanus*.

Comparison between the phylogenetic trees obtained

The Cytb phylogenetic trees for groups A and B show a consistent placement of species into two distinct clades:

- Clade 1: this clade contains species from Australasia and Asia.
- Clade 2: this clade primarily includes species from Africa, along with some species from Europe.

Determination of Haplotypes

The Cytb analysis of mitochondrial DNA conducted using DnaSP revealed significant genetic diversity. Among the 109 sequences studied, 169 polymorphic sites and 82 haplotypes were identified. Notably, 66 private haplotypes were detected, which are unique to a single population. Additionally, 16 haplotypes exhibited haplotypic diversity, each consisting of 2 to 4 identical sequences. The haplotype diversity (Hd) within the analyzed DNA sequences was exceptionally high, measured at 0.9939.

Construction of phylogenetic networks

Haplotype networks representing the evolutionary and phylogenetic relationships among the determined haplotypes were generated using the NetWork program. These networks revealed two haplogroups that correspond to the different Cytb sequences studied (Fig. 4).

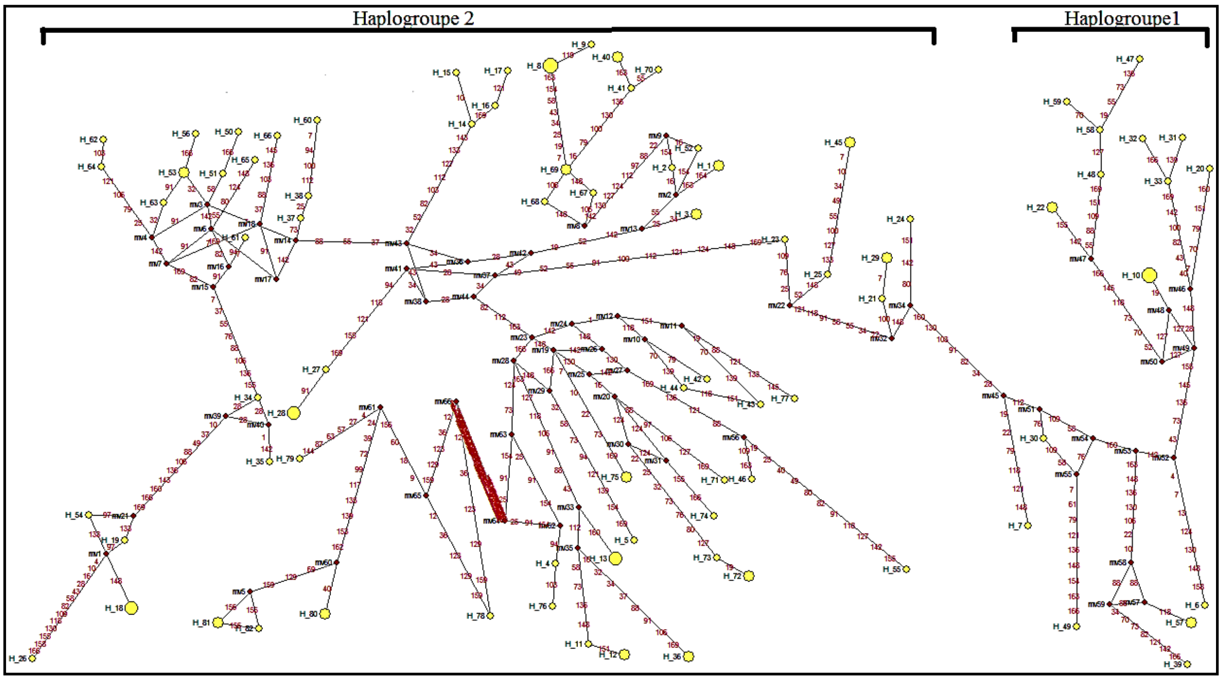

Fig. 4. Haplotypic network produced by NetWork from Cytochrome b sequences of the *Miniopterus* genus.

In the haplotype network, the size of each circle is proportional to the number of identical haplotypes observed in the dataset. The branch lengths are proportional to the number of mutations between haplotypes, with the numbers above the branches in red indicating the number of mutations. According to Saitou (1987), the greater the difference between two homologous sequences, the larger the evolutionary distance separating them, indicating they diverged longer ago. Conversely, the closer the homologous sequences, the smaller the evolutionary distance. Our analysis identified two haplogroups: Haplogroup 1, corresponding to the Australasian and Asian groups, and Haplogroup 2, corresponding to the African-European groups.

Comparison between the results of the different software used

To evaluate the effectiveness of our study, we compared the results obtained from different software tools. The construction of the phylogenetic tree of the Cytb gene using MEGA allowed us to observe a distribution of species into two clades: the Australasian-Asian clade and the African-European clade. These results were consistent with the haplotypic networks generated by the NetWork program, which also showed two major haplogroups corresponding to the same geographical distribution presented in the phylogenetic tree.

Nicole (2008) emphasized that localized analyses on haplotypic networks offer more precision, leading us to validate our results through the Cytb gene haplotypic networks. According to Saitou and Nei (1987), haplotypic diversity (Hd) is defined as the probability that two genes drawn at random from a sample are identical. This is represented in our phylogenetic tree by the placement of identical sequences within the same clade and in the NetWork program by circles proportional to the number of identical haplotypes or points for unique haplotypes.

MEGA is a comprehensive tool for statistical analysis of DNA and protein sequences, offering functionalities such as sequence alignment and phylogenetic tree construction (Tamura et *al*., 2011). NetWork, on the other hand, excels in reconstructing phylogenetic networks, estimating ancestral types, and visualizing evolutionary relationships (Forster and Forster, 2020). By using both tools, we were able to cross-validate our findings and ensure robust phylogenetic inferences.

Phylogenetic relationships between species of the genus *Miniopterus*

Our examination of the overall taxonomy among *Miniopterus* reveals two monophyletic clades, each geographically separated. According to Burland et *al.* (1999), McCracken et *al.* (1994), and Petit et *al.* (1999), this separation indicates that gene flow occurs within distinct geographic areas.

1. Phylogenetic relationships within the Australasia-Asia clade

This clade is well-defined, containing monophyletic groups distributed across Australia, New Guinea, and Asia. Notably, *M. fuliginosus* from Japan is closely related to *M. magnater*, suggesting significant differences in cranial and dental characteristics, as reported by Li et *al*. (2015). Christidis et *al*. (2014) proposed that Australian *M. schreibersii* are derived from their Spanish counterparts, leading to divergences and the emergence of new subspecies: *M. schreibersii oreanae, oceanensis, bassani,* and *blepotis*. Cardinal and Christidis (2000) highlighted the morphological distinctiveness of these subspecies. Furthermore, the close relationship between *M. macrocneme* and *M. australis* with other Asian species (*M. paululus, M. pusillus,* and *M. medius*) is affirmed by Kitchener and Suyanto (2002), indicating that these species appear as sister taxa.

2. Phylogenetic relationships within the African-European clade

This clade encompasses the majority of *Miniopterus* species, predominantly consisting of endemic Malagasy fauna, which includes a mixture of taxa from Africa and Madagascar. We identified five deep lineages among Malagasy species, each forming a monophyletic group:

D5: *M. majori, M. gleni, M. griffitsi*
D6: *M. griveaudi*
D7: *M. mahafaliensis, M. sororculus*
D8: *M. manavi, M. petersoni*
D9: *M. aelleni, M. aelleni A, M. aelleni B, M. ambohitrensis*

The species in lineage D8 are identified as sister species and morphologically distinct, linked to a group of five genetically distinct species from Madagascar (*Miniopterus sp.* Madagascar species 5, 6, 7, 8, and 9) as shown in the tree constructed by Christidis et *al.* (2014). The adaptive radiation of Malagasy *Miniopterus* is evidenced by their genetic diversity and morphological character overlap, necessitating species identification based on molecular sequence analysis.

Regarding African species, they diverge into two monophyletic groups:

Group C: contains *M. inflatus*
Group D: includes several subgroups:
D2: *M. fraterculus, M. minor*
D4: *M. africanus, M. mossambicus, M. newtonii*
D3: *M. schreibersii* (from Spain and Turkey), *M. maghrebensis, M. s. pallidus* (from Lebanon), *M. natalensis*, and the subspecies *M. s. pallidus*, suggesting they share a common ancestor despite the geographical distances.

Demos et *al.* (2019) indicated that *M. schreibersii* forms a paraphyletic species complex, with at least seven different species and subspecies in Europe, Asia Minor, and North Africa, except for the subspecies *M. s. villersi*, which is not closely related to *M. schreibersii*.

Corrected phylogenetic tree

Using the Blast bioinformatics platform on GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) allowed us to find similar regions between two or more nucleotide sequences, by comparing the regions homologs we were able to identify unclassified species nicknamed sp species in the phylogenetic trees that we obtained. The final presentation of our corrected global tree is shown in the following figure 5.

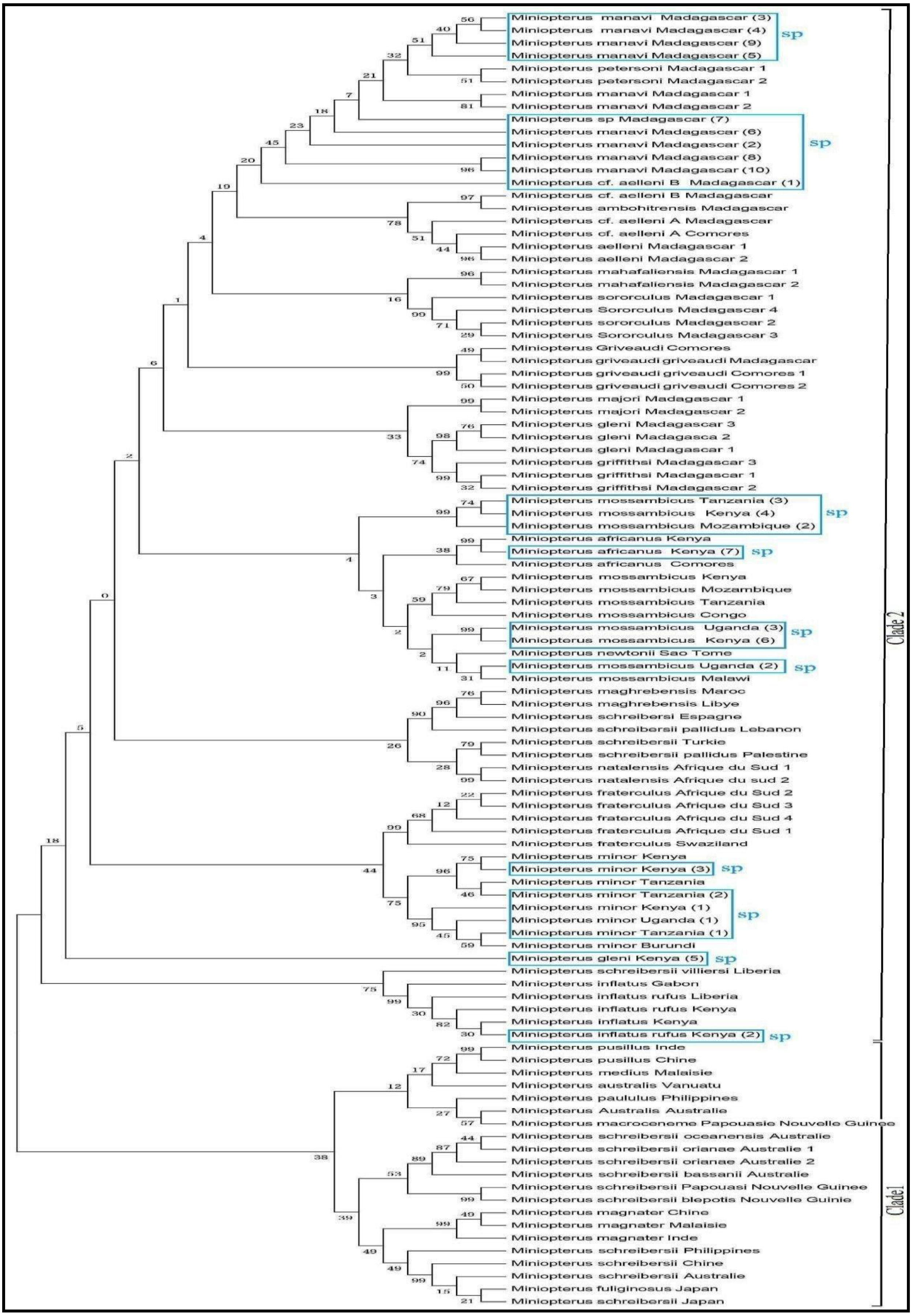

Fig. 5. Final corrected phylogenetic tree based on Cytochrome b sequences.

Miniopters in Algeria

Only one species of the *Miniopterus* genus has been determined in Algeria, it is *Miniopterus schreibersii* (Kowalski and Rzebick-Kowalska, 1991), this species is inventoried in several works including the works of Khelfaoui (2018), Bendjoudi (2019), Ahmim (2019) and Messaoud (2021). A new cryptic species found in sympatry with *Miniopterus schreibersii* was determined in North Africa, the latter seem similar for this reason the identification of the new species *Miniopterus maghrebensis* was based on molecular, cranial and dental analyzes. Molecular analyzes of *Miniopterus maghrebensis* have not been established in Algeria, but given its distribution area which extends to several regions in Morocco and Tunisia, as well as their ability to migrate to other ecological niches this species is probably present in Algeria (Puechmaille et *al*., 2014).

Conclusion

Phylogeny is an essential procedure for tracing the evolutionary history of organisms at all taxonomic levels. This analysis is also of great importance for clarifying the evolutionary patterns of species and for understanding adaptive evolution at the morphological and molecular level. Our phylogenetic study was developed using various bioinformatics software (MEGA, DnaSP, NetWork) applied to Cytb DNA sequences extracted from GenBank.

The results obtained by DnaSP to a large extent confirm the results of MEGA, whose identical sequences of each haplotype of different species correspond to the same clade in the phylogenetic tree. We also noticed that the results obtained by NetWork are comparable with MEGA, they showed the existence of two haplogroups for the Cytb gene represented by two distinct and geographically separated clades which are the African-European clade which includes the majority of species organized in monophyletic groups and distributed in the Malagasy region as well as in Sub-Saharan Africa and Europe. The second Australasian-Asia clade of monophyletic groups is distributed across regions of Australia and islands surrounding Asia. A single species *Miniopterus schreibersii* is classified in Algeria as a sister species to with European *Miniopterus schreibersii*, thus a new cryptic species *Miniopterus maghrebensis* was detected in Tunisia and Morocco, according to its distribution area we assume that the latter is present in Algeria but no work has been carried out on this subject to date.

Author’s Contributions

Author 1: Data analysis, drafting the article.

Author 2: Data analysis.

Author 3: Data analysis.

References

Ahmim M. 2019. Les mammifères sauvages d’Algérie répartition et biologie de la conservation. Les Editions du Net, 314p

Bendjoudi D., Yedou W., Beneldjouzi A., Mechouk N., Bendjeddou L. 2019. On bat ectoparasite (nyteribiidae, streblidae, siphonaptera, mesostigmata and ixodidae) from Chrea national park (central atlas mountains), Algeria. Bull. Soc. zool. Fr, 144, 2, p: 67-76.

Burland T., Barratt E., Beaumont M., Racey P. 1999. Population genetic structure and gene flow in a gleaning bat, *Plecotus auritus*. Biologir Science, 56 (5), p: 1241-1244.

Cardinal B. R., Christidis L. 2000. Mitochondrial DNA and morphology reveal three geographically distinct lineages of the large bentwing bat (*Miniopterus schreibersii*) in Australia. Australian Journal of Zoology, 48 p: 1-19.

Christidis L., Goodman S. M., Naughton K., Appleton B. 2014. Insights into the Evolution of a Cryptic Radiation of Bats: Dispersal and Ecological Radiation of Malagasy Miniopterus (Chiroptera: Miniopteridae). Plos One, 9, 3.

Demos T., Webala P., Lutz H., Kerbis J., Peterhans., Goodman S. M., Cortés-Delgado N., Bartonj M., Patterson B. 2019. Multilocus phylogeny of a cryptic radiation of Afrotropical long-fingered bats (Chiroptera, Miniopteridae). Zoologica Scripta, 49 (1), p: 1-13.

Forster P., Forster M. 2020. Network Release notes. (https://www.fluxus-engineering.com/sharenet_rn.htm).

Joffrin L. 2019. Écologie et évolution de coronavirus dans des populations des chauves-souris des îles de l'ouest de l'océan indien. Thèse de doctorat en sciences du vivant : université de la Réunion, 218p.

Huson D. H., Bryant D. 2012. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol, 23(2), p: 254-67.

Izeni P., Guillermo O., Iracilda S., Horacio S., Axel M. 2001. The Cytochrome b Gene as a Phylogenetic Marker: The Limits of Resolution for Analyzing Relationships Among Cichlid Fishes. Molecular Evolition, 53 p: 89-103.

Goodman S., Ramasindrazana B., Schoeman C. 2011. Morphological, bioacoustical, and genetic variation in Miniopterus bats from eastern Madagascar, with the description of a new species. Zootaxa, 2880 p: 1-19.

Khelfaoui F., Kebaci A., Benyacoub S. 2018. Nouvelles données sur les Insecta et les Acarina, ectoparasites des chauves-souris (Mammalia : Chiroptera) en Numidie orientale, Algérie. Bull. Soc. zool. Fr, 143(2), p: 63-73.

Kitchener D. J., Suyanto A. 2002. Morphological variation in *Miniopterus pusillus* and *M. australis* (sensu Hill 1992) in southeastern Asia, New Guinea and Australia, Records of the Western Australian Museum, 21 p: 9-33.

Kleppe K., Ohtsuka E., Kleppe R., Molineux I., Khorana H. G. 1971. Studies on polynucleotides. XCVI. Repair replications of short synthetic DNA's as catalyzed by DNA polymerases. J. Mol. Biol., 56 p: 341-361.

Kowalski K., Rzebick-Kowalska B. 1991. Mammals of Algeria. Polish Academy of Sciences. Institute of Systematics and Evolution of Animals, Wroclaw, 353p.

Li S., Sun K., Lu G., Lin A., Jiang T., Jin L., Hoyt J., Feng J. 2015. Mitochondrial genetic differentiation and morphological difference of *Miniopterus fuliginosus* and *Miniopterus magnater* in China and Vietnam. Ecologie and Evoluton, 5(6), p : 1214-1223.

Lord E. 2015. Flux de travaux et leurs applications en bioinformatique. Thèse du doctorat en informatique : université du Québec à Montréal, 4, 233.

McCracken G., McCracken M., Vawter T. 1994. Genetic structure in Migratory populations of the bat *Tadarida brasiliensis mexicana*. Journal of Mammalogy, 75 (2), p: 500-514.

Messaoud N., Derouiche L., Baha M. 2021. Étude de la dynamique des colonies du grand Rhinolophe (*Rhinolophus ferrumequinum,* Mammalia, Chiroptera) au parc national de Chréa (Algérie). Revue Agrobiologia 10(1) 29.

Meganathan P. R., Dubey B., Jogayya K. N., Haque I. 2011. Validation of a Multiplex PCR Assay for the Forensic Identification of Indian Crocodiles. J Science médico-légale; 56(5) p: 1241-4.

Miller-Butterworth C., Murphy W., O'Brien S., Jacobs D., Springer M., Teeling E. 2007. A family matter: conclusive resolution of the taxonomic position of the long-fingered bats, Miniopterus. Mol. Biol. Evol, 24(7), p: 1553-1561.

Nicole W., Steven M., William T., Manule R. 2008. The biogeography of Miniopterus bats (Chiroptera: Miniopteridae) from the Comoro Archipelago inferred from mitochondrial DNA. Molecular Ecology, 17 p: 5205-5219.

Patwardhan A., Ray S., Roy A. 2014. Molecular markers in phylogenetic studies-review. Phylogenetics & Evolutionary Biology, 2, 2.

Petit E., Excoffier L., Mayer F. 1999. No Evidence of bottleneck in the postglacial recolonization of Europe by the noctule bat (*Nyctalus Noctula*). Evolution, 53(4), p: 1247-1258.

Puechmaille S. J., Allegrni B., Benda P., Gurun K., Sramek J., Ibnez C., Just J., Bilgin R. 2014. A new species of the *Miniopterus schreibersii* species complex (Chiroptera: Miniopteridae) from the Maghreb region, north Africa. Zootaxa, 379(4) 1, p: 108-124.

Rozas J., Ferrer-Mata A., Sanchez-DelBarrio J., Guirao-Rico S., Librado P., Ramos-Onsins S., Sanchez-Gracia 2017. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Mol. Biol. Evol, 34(12), p: 3299-3302.

Saitou N., Nei M. 1987. The Neighbor-joining method: A new method for reconstructing phylogenetic trees. Neighbor-Joining method, 4, 4.

Sayers E., Cavanaugh M, Clark K., Pruitt K., Schoch C., Sherry S., Karsch-Mizrachi I. 2020. GenBank. Nucleic Acids Research, 49(10). 1093p.

Stevens R., Willig M., Richard E., Strauss., Lundberg P. 2006. Latitudinal Gradients in the Phenetic diversity of new world bat communities. Oikos, 112 p: 41-50.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution, 28(10), p: 2731-2739.

Teeling E. C., Springer M. S., Madsen O., Bates P., O'Brien S. J., Murphy W. J. 2005. A molecular phylogeny for bats illuminates biogeography and the fossil record. Science, Vol. 307, Issue 5709, p: 580-584.

Villesen P. 2007. FaBox: an online toolbox for FASTA sequences. Molecular Ecology Notes, 7 p: 965-968.

Yoann M. 2012. Les arbres phylogénétiques : construction et interprétation. Bioinf-fr.net Geekus biologicus.

Yvan S., Inaki I., Pedro L. 2007. A review of feature selection techniques in bioinformatics. Bioinformatics, 23(19), p : 2507-2517.