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# **Evolution of the Snake Superfamily Elapoidea Through the Eyes of Genomics and Phenomics**

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DOCTORAL DISSERTATION

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Dedicated in the memory of Hirendra Kumar Roy, Sujit Basu Bhowmik  
and Ashoke Ranjan Das

## Abstract

Snake superfamily Elapoidea is a cosmopolitan, diverse group containing nearly one-fifth of the global snake diversity. Members of this superfamily includes some of the most venomous snakes like cobras and mambas. It also includes both very generalist snakes and snakes highly specialised to a particular habitat, such as burrows and open oceans, and dietary habits, such as other snakes, centipedes and reptile eggs.

Despite having two groups of medically significant venomous snakes and an ecologically, behaviourally and phenotypically highly diverse assemblage of lineages, family level classification of Elapoidea remained controversial and unstable. The reason behind much confusion over elapoid systematics is the lack of consensus of the deeper level phylogenetic relationships within Elapoidea. Previous attempts to infer the phylogeny of elapoids with traditional mitochondrial and nuclear markers as well as small-scale genome-wide data sets has resulted in highly incongruent, poorly supported phylogenies. The degree of discordance has been so high that no workable consensus could be drawn.

In this thesis, I generated the largest target capture genome-wide marker dataset for elapoid snakes to date, sampling representatives of every major elapoid clade and genera with uncertain taxonomic affinities within Elapoidea. Phylogenomic analyses of this dataset yielded fully resolved phylogenies which were statistically well-supported and were mostly congruent across different combinations of target capture loci and tree inference methods. Inferred phylogenies also demonstrated *Buhome*, *Micrelaps* and *Psammodynastes* to represent unique branches within Elapoidea and I described new families to accommodate them.

Elapoidea was found to have originated in Asia in the Eocene from where they dispersed into Africa, the Americas and Australasia. Early elapoids experienced rapid diversification and the major extant groups already appeared in the Eocene. This ancient radiation has resulted in extensive incomplete lineage sorting and possibly some degree of early gene flow, making elapoid phylogeny difficult to resolve.

I also used a vast number of micro-computed tomographic scans to gather anatomical data that aided discovery of diagnostic characters for the newly described families and facilitated classification of rare genera.

## Tiivistelmä

Käärmeiden yläheimo Elapoida on kosmopoliittinen ja monimuotoinen eliöryhmä, joka sisältää lähes viidesosan maailman käärmeiden monimuotoisuudesta. Siihen kuuluu sekä maailman myrkyllisimpiä käärmkeitä (esim. kobrat ja mambat) että lajeja, jotka ovat sopeutuneet äärimmäisen erikoistuneisiin elinympäristöihin (esim. maanalaisiin tai avomeri ympäristöihin) ja ravintolokeroihin (esim. muiden käärmeiden, tuhatjalkaisten tai matelijoiden munien hyödyntämiseen ravintona). Tämän eliöryhmän heimotason luokittelu on kuitenkin säilynyt kiistanalaisena ja epävakana, vaikka siihen sisältyy kaksi lääketieteellisesti merkittävää myrkyllisten käärmeiden ryhmää sekä ekologisesti, käyttäytymiseltään ja ilmiänsuultaan erittäin monimuotoisia lajeja. Tämän epävarmuuden taustalla on yhteisymmärryksen puute syvistä fylogeneettisista suhteista ryhmän sisällä. Aikaisemmat fylogeneettiset analyysit, jotka ovat tukeutuneet perinteisiin mitokondrio- ja tumamarkkereiden hyödyntämiseen, tai pienimuotoisiin kokogenomiaineistoihin, ovat johtaneet ristiriitaisiin ja heikosti tuettuihin hypoteeseihin, mikä on vaikeuttanut konsensuksen muodostumista Elapoidae yläheimon evolutiivisesta historiasta.

Tässä väitöskirjatyössä kokosin tähän mennessä laajimman genomisen markkeriaineiston Elapoida yläheimolle kaikista sen merkittävistä sukuhaaroista sekä taksonomisesti kiistanalaisista suvuista. Fylogenomiset analyysit tuottivat tilastollisesti vahvasti tuettuja fylogeneettisiä hypoteeseja, jotka olivat yhteneväisiä riippumatta käytetyistä markkeriyhdistelmistä ja puunrakennusmenetelmistä. Tulosten perusteella pystyin päättämään, että suvut *Buroma*, *Micrelaps* ja *Psammodynastes* edustavat Elapoidae sisällä omia ainutlaatuisia evolutiivisia linjojaan, ja perustin näitä varten kolme uutta heimoa.

Elapoida yläheimon havaittiin syntyneen Aasiassa eoseenikaudella, mistä se levisi Afrikkaan, Amerikkoihin ja Australaasiaan. Ryhmän varhaiset edustajat kävivät läpi nopean monimuotoistumisen, ja suurimmat nykyiset sukuhaarat olivat muodostuneet jo eoseenikaudella. Tämä nopeasti edennyt muinainen erilaistuminen on johtanut laajaan epätäydelliseen linjajärjestäytymiseen joka yhdessä mahdolliseen varhaisen lajien välisen geenivirran kanssa selittää sen, miksi Elapoidae yläheimon fylogeneettiset sukulaissuhteet ovat olleet vaikeasti ratkaistavissa.

Työssäni hyödynsin myös mikro-tietokonekerroskuvantamista kerätäkseni anatomista tietoa, joka auttoi uusien heimojen diagnostisten piirteiden määrittämisessä ja harvinaisten sukujen luokittelussa.

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## List of original articles

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Article **II**: **Das, S.**, Greenbaum, E., Brecko, J., Pauwels, O. S., Ruane, S., Pirro, S., & Merilä, J. (2024). Phylogenomics of *Psammodynastes* and *Buhoma* (Elapoidea: Serpentes), with the description of a new Asian snake family. *Scientific Reports*, 14(1), 9489.

Article **III**: Weinell, J. L., Burbrink, F. T., **Das, S.**, & Brown, R. M. (2024). Novel phylogenomic inference and ‘Out of Asia’ biogeography of cobras, coral snakes and their allies. *Royal Society Open Science*, 11(8), 240064.

Article **IV**: **Das, S.**, Weinell, J. L., Konstantopoulou, A., Brown, R. M., Burbrink, F. T., Brecko, J., Pauwels, O. S. G., Greenbaum, E., & Merilä, J. (2025). Megaphylogenomics and revised classification of Elapoidea, with description of a new African family. *Manuscript*.

Article **V**: **Das, S.\***, Brecko, J.\*, Pauwels, O. S., & Merilä, J. (2022). Cranial osteology of *Hypoptophis* (Aparallactinae: Atractaspididae: Caenophidia), with a discussion on the evolution of its fossorial adaptations. *Journal of Morphology*, 283(4), 510-538.

\*These authors contributed equally

### Author contributions

	Article I	Article II	Article III	Article IV	Article V
Concept	SD	SD	JLW, FTB, SD, RMB	SD, JLW, FTB, RMB, JM	SD
Analyses	SD	SD	JLW	SD, AK	SD, JB
Manuscript preparation	SD, EG, SM, AMB, FTB, CJR, JLW, RMB, JB, OSGP, NR, APR, JM	SD, EG, JB, OSGP, SR, SP, JM	JLW, FTB, SD, RMB	SD, JLW, AK, RMB, FTB, JB, OSGP, EG, JM	SD, JB, OSGP, JM



# 1. Introduction

A fully resolved and stable phylogeny is an indispensable prerequisite of systematics, evolutionary biology and various branches of comparative biology. Lack of a robust phylogeny keeps the taxonomy in a constant state of flux, impeding scientific communications and creating confusion for conservation biologists, legislators and policy makers. It also hampers research to understand the evolution of toxins and venom delivery systems for venomous organisms. Despite containing more than one-sixth of global snake diversity, the phylogenetic history of superfamily Elapoidea has remained poorly resolved. In my thesis, I aim to infer a well-supported phylogeny of Elapoidea using target capture sequencing data to stabilise the classification of Elapoidea. I also strive to estimate the time and geographic location of diversification of elapoid snakes and investigate the underlying causes of discordance. Using non-invasive imaging, I look for diagnostic features. Articles **I**, **II**, **III** and **IV** focus on phylogenomics, divergence time, biogeography and confounding factors, whereas the Article **V** focuses on cranial anatomy one particularly enigmatic elapoid taxon.

## 1.1. Systematics of Elapoidea

Phylogenetic systematics of snakes has become considerably better understood and stable in the 21<sup>st</sup> century courtesy to a vast body of phylogenetic research (e.g., Vidal *et al.* 2007; Zaher *et al.* 2009, 2019; Pyron *et al.* 2011, 2013, 2014; Streicher & Wiens 2016; Zheng & Wiens 2016, to cite a few). However, a few grey areas have remained in the otherwise well-resolved snake phylogeny and arguably the most controversial and unstable major group has been the superfamily Elapoidea.

With over 760 species belonging to the family group taxa assigned to the superfamily, this rather major clade constitutes nearly one-fifth of the global extant snake diversity (Reptile Database [Uetz *et al.* 2025]). Snakes assigned to this superfamily have a cosmopolitan distribution in Africa (including the island of Madagascar), Asia, southern Europe, both Americas, the Oceania and the oceans (Glaw & Vences 2007; Kelly *et al.* 2009; Das 2015; Geniez 2018; O'Shea 2018; Spawls *et al.* 2018; Jackson & Chiappaux 2019; Bar *et al.* 2021; Eiper & Eiper 2022; Marais 2022).

As its name implies, the well-known Elapidae family of front-fanged venomous snakes is a member of this superfamily (Figure 1). Elapids include some of the most venomous terrestrial

and marine snakes, such as the cobras, mambas, kraits, coral snakes, tiger snakes, taipans and sea snakes (O'Shea 2005; Spawls & Branch 2020). Elapids are distributed in all the continents (except Europe), two oceans and forms the bulk of the Australian snake fauna. Another group of rather odd, African and middle eastern venomous snakes, capable of delivering a laterally stabbing bite without even having to open their mouth, burrowing asps are also a member of Elapoidea. They are classified either as family Atractaspididae with no subfamilies (e.g., Kelly *et al.* 2009) or two subfamilies (e.g., Zaher *et al.* 2019) or as a subfamily of Lamprophiidae (e.g., Pyron *et al.* 2013). Several African genera closely related to burrowing asps, often classified in recent literature as subfamily Aparallactinae of Atractaspididae (e.g., Zaher *et al.* 2019; Portillo *et al.* 2018, 2019) or Lamprophiidae (e.g., Pyron *et al.* 2013; Figueroa *et al.* 2016), also includes poorly known rear-fanged, mostly fossorial snakes with unusual diets, such as centipede eating. African shovel-snouts, assigned to family Prosymnidae (e.g., Kelly *et al.* 2009; Zaher *et al.* 2019) or subfamily Prosymninae under Lamprophiidae (e.g., Pyron *et al.* 2013), are yet another group of fossorial snakes that specialise on an unusual diet of reptile eggs (Spawls *et al.* 2018; Heinicke *et al.* 2020). The elegant, fast-moving sand snakes and Montpellier snakes of Africa, Asia, middle east and Europe also belong to Elapoidea, classified as family Psammophiidae (e.g., Kelly *et al.* 2009; Zaher *et al.* 2019) or as a subfamily of Lamprophiidae (e.g., Pyron *et al.* 2013; Figueroa *et al.* 2016). Elapoids also includes a clade of Malagasy gem snakes and African slug eaters, classified as Pseudoxyrhopiidae (e.g., Kelly *et al.* 2009; Zaher *et al.* 2019) or subfamily Pseudoxyrhopiinae under Lamprophiidae (e.g., Pyron *et al.* 2013; Burbrink *et al.* 2019), that forms the bulk of the ophidiofauna of Madagascar. Pseudaspidae (e.g., Kelly *et al.* 2009; Zaher *et al.* 2019), also regarded to be a subfamily Pseudaspidinae under Lamprophiidae (e.g., Pyron *et al.*, 2013), contains two rather dissimilar snakes native to southern Africa. Another major, quite variable, African group of non-venomous house snakes, wolf snakes, file snakes etc are assigned to Lamprophiidae, with no subfamilies (e.g., Kelly *et al.* 2009; Zaher *et al.* 2019) or to Lamprophiinae under a broad Afro-Malagasy Lamprophiidae (e.g., Pyron *et al.* 2013). Recently, some Philippines-endemic genera were demonstrated to form a monophyletic cluster within Elapoidea and a new subfamily Cyclocorinae under Lamprophiidae was established by Weinell & Brown (2018). However, the original authors elevated this subfamily to family rank in a subsequent publication (Weinell *et al.* 2020). Three rear-fanged genera – Asian viper-mimic mock vipers (*Psammodynastes*), sub-Saharan African *Buhoma* and African and middle eastern *Micrelaps*, are also assigned to Elapoidea with uncertain affinities to recognised families/subfamilies.

This brief introduction into the diversity of elapoid snakes already gives a clear hint to a major issue – a highly unstable classification scheme. Different higher level classification schemes are often subjective choices made by systematists. That, however, is not at all the case with Elapoidea. Strong disagreement on this topic stems from a mostly unresolved deeper level phylogeny. The monophyly of Elapoidea has generally been uncontroversial (e.g., Vidal & Hedges 2002; Nagy *et al.* 2005; Vidal *et al.* 2007; Vidal *et al.* 2008; Kelly *et al.* 2009; Pyron *et al.* 2011, 2013, 2014; Figueroa *et al.* 2016; Zheng & Wiens 2016; Zaher *et al.* 2019). However, the phylogenetic relationships between the major subclades within Elapoidea, treated variously as families or subfamilies, have been extremely incongruent between studies. It can already be noticed from the introductory paragraph on elapoid diversity that the predominantly Afro-Malagasy clades are sometimes treated as subfamilies under a broad Lamprophiidae. This is usually done by authors whose analyses recover a monophyletic Afro-Malagasy radiation (Pyron *et al.* 2011, 2013, 2014; Zheng & Wiens, 2016). On the other hand, studies inferring trees where Afro-Malagasy groups are not monophyletic with respect to Elapidae propose and favour a multi-family classification, restricting Lamprophiidae only to a smaller assemblage of African genera (e.g., Kelly *et al.* 2009; Zaher *et al.* 2009, 2019). However, it should not be assumed that there exists any degree of phylogenetic congruence beyond the monophyly, or lack thereof, of Afro-Malagasy clades amongst studies proposing or using the same classification. The topology of Kelly *et al.* (2009), for instance, is very different from that in Zaher *et al.* (2019). An attempt to generate a consensus phylogeny from all the studies would essentially result in a star-tree and a consensus of studies adopting similar classification would also consist of mostly polytomies. It is noteworthy that all the cited studies had very poor statistical support for deep elapoid branches. Smaller scale phylogenomic ('phylogenomics' is used in this thesis strictly to mean studies using >100 loci generated with next generation sequencing approaches) studies by Pyron *et al.* (2014) and Burbrink *et al.* (2020) lacked taxa required for a comprehensive phylogeny and classification. They also suffered from poor branch support.

Distribution on multiple continents makes Elapoidea an excellent system for biogeographic research. Elapoidea also contains two groups of medically significant venomous snakes and several rear-fanged and completely non-venomous taxa. A comprehensive understanding the evolution of complex venom delivery systems and venom proteins will never be possible without studying these processes in elapoids. Last but not the least, a stable classification scheme congruent with the evolutionary history is the basic necessity of comparative biology

and scientific communication on biodiversity. However, all of these require a well resolved, statistically supported and stable phylogeny. The existing phylogenetic research could not provide any of that for Elapoidea.



Figure 1: Spectacled cobra (*Naja naja*) – an iconic member of Elapoidea. Image credit – Ville-Veikko Kivisaari.

## 1.2. Ancient rapid radiation

What makes the elapoid phylogeny so hard to resolve? The answer possibly lies in how the early elapoid lineages diversified. Kelly *et al.* (2009) was the first to suggest that major clades within Elapoidea arose via a series of rapid diversifications in the Eocene. Amidst the extreme topological incongruence, something that all the phylogenetic studies published since the seminal paper of Kelly *et al.* (2009) agrees on is the presence of very short branches (in both substitution- and time-scaled trees) at the base of the elapoid phylogeny. This is a clear signature of an ancient rapid radiation, one of the most recalcitrant scenarios to resolve in evolutionary biology (Rokas & Carroll 2006; Whitfield & Lockhart 2007; Whitfield & Kjer 2008). Fast cladogenesis, resulting in short branches, means there will be little phylogenetic information content on those branches to aid in resolution. Incomplete lineage sorting will be especially likely on such short branches (Degnan & Rosenberg 2009). A rapid radiation taken

place in deep past will have the additional problem of substitutional saturation on descendant branches that will swamp the already feeble phylogenetic signal on the short ‘rapid radiation phase’ ancestral branches (Whitfield & Lockhart 2007). Fast diverging early lineages might have attained very little reproductive isolation and are not unlikely to have experienced interspecific gene flow. All of these factors have indeed been found to confound the attempts to resolve rapid radiations in both plants and animals (e.g., Longo *et al.* 2017; Cloutier *et al.* 2019; Guo *et al.* 2021; Esquerre *et al.* 2022).

## 2. Aims of the thesis

The primary objectives of this thesis were the following:

1. Inferring a stable, consistent (across different tree inference methods), statistically well supported phylogeny of Elapoidea.
2. Proposing a higher-level classification scheme fully congruent with the phylogenetic hypothesis
3. Investigate the phylogenetic relationships of *Buhoma*, *Micrelaps* and *Psammodynastes* and if required, establish new family group taxon for them.

Secondary aims of the thesis included:

4. Inferring the timing and biogeography of elapoid diversification
5. Identifying the confounding factors making the reconstruction of the elapoid phylogeny difficult
6. Finding morphological diagnostic characters for major elapoid clades and determine phylogenetic affinities for genera lacking any molecular data.

### 3. Materials and methods

This section summarises the methodology used in Articles **I**, **II**, **III**, **IV** and **IV**. Details on the used samples and methods can be found in the materials and methods section of the individual articles.

#### 3.1. Samples

**3.1.1. Tissue and genomic DNA samples:** Tissue and genomic DNA samples used in the original articles of this thesis came from the collection of natural history museums, namely American Museum of Natural History (USA), California Academy of Sciences (USA), Field Museum of Natural History (USA), Harvard University Museum of Comparative Zoology (USA), Steinhardt Museum of Natural History (Tel Aviv University, Israel), University of Kansas Biodiversity Institute (USA), University of Texas at El Paso Biodiversity Collections (USA) and Villanova University (USA). Samples include both general holdings of the named museums and research tissue collections of the collaborators accessioned to the same institutes.

**3.1.2. Non-invasive imaging samples:** Preserved specimens used for micro-computed tomographic ( $\mu$ -CT) scans generated for the articles in the present thesis came from Royal Belgian Institute of Natural Sciences (Belgium), Royal Museum of Central Africa (Belgium) and Steinhardt Museum of Natural History (Tel Aviv University, Israel).

#### 3.2. Generation and processing of genomic and anatomical data

**3.2.1. Genomic DNA extraction and sequencing:** Wet laboratory works for the included articles were conducted at University of Helsinki, University of Texas at El Paso, University of Kansas Biodiversity Institute and Field Museum of Natural History. Genomic DNA extraction was carried out manually using Macherey Nagel NucleoSpin Tissue kit (Article **I**) and Qiagen DNeasy Blood and Tissue kit (Article **II**) or with Maxwell Rapid Sample Concentrator Instrument using Maxwell16 Tissue DNA Purification Kit (Article **III**). Quality checks and quantification were done with 1% agarose gel electrophoresis, Qubit and Nanodrop.

Genomic DNA samples used in Article **I** were outsourced to RAPiD Genomics (USA) for enrichment for ultraconserved elements (UCEs) with Tetrapods- UCE-5Kv1 probe set (Faircloth *et al.* 2012) and Illumina sequencing. The UCE enrichment kit can target up to 5060 UCE loci in tetrapod genomes. A different enrichment probe set, MyBaits-20 custom RNA kit (Arbor BioSciences, USA), targeting 1652 rapidly evolving exons (REEs), 907 UCEs, 328

ddRAD-like, 27 major histo-compatibility, 119 vision-associated and 95 scalation-associated (a total of 3128) loci was used to generate the data used in Article **III**. Target enrichment was performed by Arbor BioSciences followed by Illumina HiSeq X sequencing at NovoGene (USA).

To generate the reference genome of *Psammodynastes pulverulentus* for Article **II**, library preparation was carried out using Illumina TruSeq kit with standard adapters on genomic DNA extracts from FMNH 273629 in the collection of the Field Museum of Natural History. Sequencing was done on an Illumina X-Ten platform at 105X coverage. Wet laboratory procedures were done by Iridian Genomes (USA).

Traditional mitochondrial markers, namely cytochrome B (*CYTB*), NADH dehydrogenase subunit 4 (*ND4*) and 12S and 16S ribosomal RNA genes (*12S* and *16S*), and nuclear markers, namely brain-derived neurotrophic factor (*BDNF*), oocyte maturation factor (*C-MOS*), 3'-nucleotidase (*NT3*) and recombination activating gene 1 and 2 (*RAG1* and *RAG2*), were used in Articles **II** and **III** to allow for representation of taxon. Amongst the aforementioned markers, *CYTB*, *ND4*, *16S*, *BDNF*, *C-MOS*, and *RAG1* were specifically sequenced for Article **I** and **III** and *12S* gene, *NT3* and *RAG2* sequences used in Article **I** were obtained from GeneBank. Most primers used are from Burbrink *et al.* (2000), Lawson *et al.* (2005), Noonan & Chippindale (2006), Portillo *et al.* (2019), Weinell *et al.* (2020). Additionally, a pair of new forward and reverse *CYTB* primers were designed with Primer3 v0.4.0 (Untergasser *et al.* 2012) in Article **III**. Number polymerase chain reaction (PCR) cycles performed were 32 (only for mitochondrial markers used in Article **II**) and 34. Amplified PCR products were visualised by running 1.5% agarose gel electrophoresis. PCR product purification and Sanger sequencing were carried out at the University of Texas at El Paso Genomic Analysis Core Facility and GeneWiz (USA) for Articles **II** and **III** respectively.

**3.2.2. Processing of genomic data:** Phyluce pipeline (Faircloth 2016) was used to process the raw sequencing reads generated for Article **I**. Illumiprocessor, a Trimmomatic-based (Bolger *et al.* 2014) tool, was used to trim the adapters. SPAdes (Bankevich *et al.* 2012) assembler implemented in phyluce was then used to assemble the trimmed reads into contigs. In the next step, UCE loci in the contigs were identified with the Tetrapods- UCE-5Kv1 probe set. The loci found in this step are then extracted and aligned with the MAFFT aligner (Katoh & Standley 2013) available in the same pipeline. Finally, three aligned UCE datasets with 50%, 75% and 95% completeness were generated.

Phyluce pipeline was also used to generate the dataset for Article **II**, albeit in a different capacity. Article **II** combines the UCE data from Article **I** with *in silico* captured UCEs from available elapoid and outgroup reference genomes. Of those reference genomes, the one of *Psammodynastes pulverulentus* was generated specifically for Article **II**. Raw reads of this sample were first assembled into scaffolds using SPAdes followed by genome finishing with in-house Zanfona pipeline developed by Iridian Genomes (USA). The other reference genomes were obtained from NCBI Genome database. Phyluce pipeline was then used to find the UCE loci in the reference genomes with Tetrapods- UCE-5Kv1 probe set. The identified and extracted UCE loci were then combined with Article **I** dataset followed by realignment with MAFFT and generation of 50%, 75%, and 95% complete datasets.

A different approach was adopted to process the raw target-capture reads generated in Article **III**. BBDMap (Bushnell 2014) and FASTP (Chen *et al.* 2018) were used to demultiplex, and adapter trim the raw reads respectively. BBDMap was then used to remove contaminants, and duplicate reads were removed with 'dedupe' implemented in BBTools. Read pairs were merged with BBMerge (Bushnell *et al.* 2017). SPAdes was used to assemble the reads. Low complexity regions and repeats were masked with repeatMasker (Smit *et al.* 2013). BLASTn, R package igraph (Csardi & Nepusz 2006) and SeqKit 2 (Shen *et al.* 2016) were used to match contigs to MyBaits-20 custom RNA kit probes, filter matches with bit-score < 50 and cluster the disjointed contigs.

Article **III** also leveraged the available reference genomes for a denser taxon sampling. The approach to *in silico* harvest the target loci, however, differed from the one adopted in Article **II**. Consensus sequences was generated with R package Biostrings (Pagès *et al.* 2021) using MAFFT alignments of *in vitro* target capture loci. BLASTn, with bit-score > 50, was used to identify the matching regions in the reference genomes. Loci that matched multiple regions in a genome were excluded. Finally, the newly sequenced and *in silico* harvested loci were aligned with MAFFT.

Article **IV** utilised the available UCE datasets of Articles **I** and **II**, mixed loci target capture dataset of Article **III** and SqCL dataset of Title *et al.* (2024). SqCL is a probe set designed to enrich UCEs, anchored hybrid enrichment loci (AHE) and some traditionally used markers (Singhal *et al.* 2017). Of these, Article **IV** used the UCE and AHE loci, which also constitute the bulk of the SqCL data. The UCEs in the SqCL uses the same loci indexing system as the Tetrapods- UCE-5Kv1 probe set, straightforwardly allowing combining of Articles **I**, **II** and

Title *et al.*'s (2024) UCE data. However, the UCE loci indexing convention in Article **III** was different from the Tetrapods- UCE-5Kv1 probe set. Moreover, there are possibilities of overlap between the REE, ddRAD-like and various trait-associated loci with AHEs and UCEs. However, attempts to identify the homologues of Article **III** UCE markers in the other datasets and exploration of overlap between different types of loci with default BLASTn searches without gap opening and extension penalties and a required level of percent identity invariably returned biologically meaningless matches (determined by visual examination in MEGA 11 (Tamura *et al.* 2021) and UGENE (Okonechnikov *et al.* 2012). BLASTn search with an e-value of  $1e-50$ , gap opening and extension penalty of 5 and percent identity of 85 in both directions (i.e., alternating the query and the subject) allowed for identification of biologically meaningful overlaps between different types of loci among the reciprocal matches. Visual examination of putative matches found in only one direction of BLASTn searches invariably turned out to be artefactual matches between non-homologous loci. To determine the homology relations between the UCEs from Article **III** and the rest of the articles (including Title *et al.* 2024), BLASTn searches were carried in the same way as above. However, to ensure no redundancy in UCE loci, BLASTn searches were also performed with more relaxed e-value ( $1e-25$ ) and percent identity (75 and 65) parameters but this did not increase the number of matches. All reciprocal UCE matches were found to be homologous loci. All the matches found in only one search direction were also checked visually and some of them were found to be homologues. Majority of the matches that were not reciprocal, however, were artefactual. The AHE and UCE loci overlapping with the REE, ddRAD-like and trait-associated loci were excluded. The UCE loci from Article **III** and combined Articles **I**, **II** and Title *et al.* (2024) dataset found to be homologous were appended together and the rest were analysed separately. Finally, all the loci were realigned with MAFFT and alignment of > 500 loci were visually inspected.

To assemble raw Sanger sequencing reads, Article **II** used Pearl programme from Tracy (Rausch *et al.* 2020) whereas Article **III** used GENEIOUS 6.1. MAFFT was used to align the genes.

**3.2.3. Micro-computed tomographic data:** Micro-computed tomographic ( $\mu$ -CT) scans were done on the head of preserved museum specimens with GE Phoenix Nanotom S (GE Measurement and Control Solutions) at the University of Helsinki and EasyTom 150 (RX Solutions) and XRE UniTom (Tescan XRE) at the Royal Belgian Institute of Sciences and the Royal Museum of Central Africa. Volume rendering was done with Dragonfly (Object

Research System Inc.) and 3D meshes were visualised with MeshLab (Cignoni *et al.* 2008). Additional elapoid scans were obtained from DigiMorph and MorphoSource databases.

### 3.3. Analyses

**3.3.1. Phylogenomic analyses:** In all the articles, Maximum Likelihood (ML) estimates of individual gene trees were done with IQ-TREE 2 (Minh *et al.* 2020a). ModelFinder (Kalyanamoorthy *et al.* 2017) was used to choose the best model for each locus. Ultrafast bootstrapping (UFBoot; Hoang *et al.* 2018) was performed with 1000 replicates. ASTRAL (Mirarab *et al.* 2014) and weighted ASTRAL (wASTRAL; Zhang & Mirarab 2022), which has been demonstrated to be more accurate than unweighted ASTRAL, were used to infer multispecies coalescent phylogenies from ML gene trees. For wASTRAL, three different weighting schemes using branch length, branch support or both (hybrid), were used. Of these, wASTRAL-hybrid is the most accurate. Branch support is measured with local posterior probability (LocalPP).

Article **IV** used a newly developed multispecies coalescent tree inference method CASTER (Zhang *et al.* 2025). This method directly uses the sequence data, is highly scalable and does not make any assumption of recombination-free locus. CASTER-Site works with individual sites under a Hasegawa-Kishino-Yano (HKY) model whereas CASTER-Pair works with pairs of sites under a General Time Reversible (GTR) model. CASTER uses local block (10000 bp) bootstrap (LBBot) as a measure of branch support. Both implementations of CASTER, especially the CASTER-Pair, have been demonstrated by Zhang *et al.* (2025) to be more accurate than several other gene tree and sites-based coalescent tree inference methods and ML concatenation methods. A supermatrix of all the loci was used as an input in CASTER-Site and CASTER-Pair analyses in Article **IV**.

ML phylogenies from concatenated supermatrix of loci were also inferred with IQ-TREE 2 in Articles **I**, **II** and **III**. ModelFinder was used to choose the best partitioning scheme and models. However, the MFP+MERGE option proved to be computationally infeasible and TESTMERGE option that functions like PartitionFinder (Lanfear *et al.* 2012) was used instead. Branch support was determined with 1000 replicates of UFBoot. Article **II** used GENESITE sampling strategy for bootstrapping to reduce the chances of wrongly inferred branches receiving high support. Besides UFBoot, Articles **I** and **III** also used Shimodaira Hasegawa-like approximate likelihood ratio test (SH-*alrt*; Guindon *et al.* 2010) as an additional branch support metric.

Article **II** also inferred a Bayesian Inference (BI) inference phylogeny from the concatenated dataset with ExaBayes 1.5.1 (Aberer *et al.* 2014). ExaBayes was ran on unpartitioned datasets with 200 MPI (message passing interface) processes. Two independent runs with three heated and one cold chain were performed, with sampling every 500 generations. Though the target number of generations (100M) could not be reached even with two consecutive runs on the cluster, the average standard deviation of split frequencies fell below 5% which is indicative of a good degree of convergence.

To test for presence of horizontal (hybridisation/introgression) edges in the elapoid tree, Articles **II** and **III** used Maximum Pseudolikelihood-based (MPL) multispecies coalescent network inference (Yu & Nakhleh 2015; Solís-Lemus & Ané 2016) implemented in PhyloNetworks (Wen *et al.* 2018) and PhyloNet (Solís-Lemus *et al.* 2017). Both methods take inferred gene trees as input. However, while the MPL approach in PhyloNetworks uses rooted triplets induced by locus trees to infer the network, PhyloNet MPL uses quartet concordance factors computed from the input gene trees. Articles **II** and **III** inferred MPL networks with 1–6 and 1–10 permissible reticulation events, respectively. The best networks were identified on the basis of log-likelihood values or using Djump algorithm implemented in R package capushe 1.1.1 (Arlot *et al.* 2016).

Articles **I**, **II** and **III** estimated gene and site concordance (gCF and sCF) factors (Minh *et al.* 2020b). Additionally, Article **III** carried out  $\chi^2$ -tests (significance at the 0.05% level) between gene and site discordance factors (gDF and sDF). If the number of gene trees or sites supporting alternative resolutions are not significantly different in the test, the underlying discordance can be attributed to incomplete lineage sorting (ILS). The concordance and discordance factors were calculated with IQ-TREE 2.

Article **IV** used a different, new approach PhytoP (Shang *et al.* 2024) that uses detailed annotations produced by ASTRAL and wASTRAL to detect signatures of ILS and/or gene flow. Specifically, PhytoP uses the proportions (q1, q2 and q3) of rooted triplets induced by gene trees around a branch of the rooted species tree. Under an ILS only scenario, the three proportions should be very similar (i.e., roughly  $q1 = q2 = q3$ ) whereas  $q2 > q3$  would be the case if gene flow is involved. PhytoP was used to compute indices for ILS (ILS-i) and gene flow (IH-i) along with visualisation of q1, q2 and q3 at the nodes of the input rooted and annotated wASTRAL topology.

Article I also employed a test of hard polytomy using ASTRAL. The analysis tests if there is equal support for the alternative resolutions of a topology while taking ILS into account (Sayyari & Mirarab 2016).

To test if alignment or gene tree estimation errors played a misleading role in the inference of elapoid phylogeny, R package PhylteR (Comte *et al.* 2023) was used in Article IV. PhylteR can identify outlier branches in individual gene trees, entire outlier gene trees or entire outlier species and is not susceptible to ILS. PhylteR was run on ML gene trees used as wASTRAL input. After pruning the gene tree branches marked as outlier by PhylteR, wASTRAL re-run to check if this brings about any change in the topology.

**3.3.2. Timetree inference:** RelTime, a highly scalable ML-based method with accuracy comparable to computationally intensive Bayesian approaches (Tamura *et al.* 2012, 2018; Tao *et al.* 2020), was used to infer all the time-calibrated phylogenies. Articles I and II used concatenated UCE and UCE+traditional nuclear marker datasets respectively along with wASTRAL topology. Three fossil-based node calibrations, namely *Coluber cadurci* from the Oligocene and *Naja romani* and *Incongruelaps iteratus* from the Miocene (Head *et al.* 2016), were used as lognormal probability densities. RelTime analyses were run with GTR+G+I set as the substitution model.

Article III uses one dataset consisting of 10000 sites which were randomly sampled (without replacement) from the target capture data and another dataset comprising of Sanger-sequenced markers. The used calibrations, set as lognormal densities, included Miocene *I. iteratus*, *Paleoheterodon tiheni* and *Vipera antiqua* plus two indeterminate elapid and colubrid fossils from Oligocene and Eocene respectively.

Running RelTime on the full dataset of Article IV and a 5M sites subsample proved to be computationally infeasible. Therefore, a 1M sites subsample was used to infer the timetree. The tree calibrations from Articles I and II were used. Dense taxon sampling allowed for additional calibration, namely a Miocene *Bungarus* sp. All the calibrations were used as lognormal densities.

As RelTime could only be run on a subsample of the full dataset in Article IV, an alternative timetree was estimated with the branch length version of RelTime. The wASTRAL species tree branches were scaled to substitution unit from coalescent unit using CASTLES (Tabatabaee *et al.* 2023), a coalescent-aware algorithm that uses the gene trees (in this case, the IQ-TREE loci trees) to estimate species tree branch lengths with an accuracy exceeding that of ML

concatenation approaches. The same four lognormal calibration densities used for 1M site timetree were also used to time scale the wASTRAL topology with CASTES branch lengths.

**3.3.3. Biogeographic analyses:** Articles **III** and **IV** conducted historical biogeographic reconstructions. The R package BioGeoBEARS (Matzke 2013, 2014) was used for biogeographic modelling. Reconstructions under six models, namely Dispersal-Extinction-Cladogenetic or DEC, BAYAREALIKE and DIVALIKE models with and without the jump (+J) parameters, were carried out. Geographic distributions of the included elapoid taxa were coded for presence or absence in five physiogeographic terrestrial regions, namely Africa (including Madagascar and the Arabian plate), Americas, Asia (including Sunda shelf and Phillipines), Australasia (including Sahul shelf and southwestern Pacific islands), Europe (boundary with Asia corresponding to the location of early Cenozoic Turgai Strait) and a single marine region consisting of the Indian and the Pacific Oceans. Article **III** used the Sanger timetree as the input for the biogeographic reconstructions whereas the 1M sites timetree was used in Article **IV**. The best model was selected on the basis of Akaike Information Criterion and likelihood ratio test.

**3.3.4. Morphology:** Qualitative comparisons between the  $\mu$ -CT scanned crania were done. Terminology follows Cundall & Irish (2008), McDowell (2008), Palci *et al.* (2017) and Das & Pramanick (2019). A 61-character data matrix was coded for Article **V**. Maximum Parsimony (MP) and Bayesian Inference (BI) phylogenies were inferred with PAUP 4\* (Swofford 2003) and MrBayes 3.2.6 (Ronquist *et al.* 2012) respectively. The BI analysis used Markov k-state model of Lewis (2001).

## 4. Results and discussion

This section summarises the findings and conclusions made in Articles **I**, **II**, **III**, **IV** and **V**. Articles **I** and **II** infer UCE phylogenies and describes two new families, namely Microelapidae and Psammodynastidae. Article **III** reconstructs the biogeographic history and shows that Elapoidea has an Asian origin. Article **IV** infers mixed target capture dataset phylogenies, proposes a new family for genus *Buhoma* and reconfirms its biogeographic origin. Elapoidea is likely to be an Eocene rapid radiation. Deep coalescence and low phylogenetic signal are the main confounding factors, along with a probable contribution of ancient introgression. Article **V** focuses on the cranial osteology and systematics of *Hypoptophis* which is found to be an aparallactines.

### 4.1. Phylogeny of Elapoidea

Articles **I**, **II**, **III** and **IV** sampled 37, 38, 26 and 87 genera of elapoid snakes with target capture sequencing approach. The number of loci in 50%, 75% and 95% complete UCE datasets in Article **I** were 4561 (~5.6M bp), 4372 (~5.5M bp) and 3600 (~4.5M bp). The number of loci in 50%, 75% and 95% complete UCE datasets in Article **II** were 4514 (~5.3M bp), 3993 (~4.7M bp) and 2467 (~2.9M bp). Article **III** dataset had 3066 (~15.3M bp). Article **IV** dataset had 7367 (~14.6M bp). Besides the ingroup elapoid genera, Articles **I**, **II**, **III** and **IV** had 3, 4, 27 and 11 outgroup genera belonging to Acrochordidae, Boidea, Calamariidae, Colubridae, Dipsadidae, Grayiidae, Homalopsidae, Natricidae, Pareidae, Pseudoxenodontidae, Pythonidae, Sibynophiidae, Viperidae and Xenodermatidae.

Articles **I** and **II**, both using UCE datasets, reported phylogenies highly congruent and consistent across inference methods and degree of dataset completeness. The ASTRAL (used only in Article **I**) and w-ASTRAL (hybrid, branch length and branch support weighting schemes) multispecies coalescent species trees in both of these studies were fully congruent with each other (Figure 2; Figure 1 of Article **I** and Article **II**). The multispecies coalescent trees in both papers recovered Cyclocoridae as the most basal split within Elapoidea. The species tree in Article **II**, which sampled *Psammodynastes pulverulentus*, found this genus to also represent a rather early diverging lineage that is sister to Elapoidea and the Afro-Malagasy radiation. *Micrelaps muelleri* was recovered as the earliest diverging branch within the monophyletic Afro-Malagasy radiation. The rest of the Afro-Malagasy group consists of six families (classified at the subfamily level in Articles **I** and **II**) of which Malagasy Pseudoxyrhopiidae represents the basalmost split. Interestingly, Atractaspidinae, traditionally

understood to be comprised of *Atractaspis* and *Homoroselaps* (e.g., Pyron *et al.* 2013; Portillo *et al.* 2018, 2019), and Aparallactinae, i.e., all the atractaspidines barring *Atractaspis* and *Homoroselaps*, were not recovered as reciprocally monophyletic. Atractaspididae and Psammophiidae were found to be sister clades which in turn was sister to a clade comprising of Lamprophiidae, Prosymnidae and Pseudaspididae. In the coalescent species trees, prosymnids and pseudaspidids were invariably found to be sister to each other. All the family group taxa were found to be monophyletic with strong support (localPP > 0.95). The entire backbone of the elapoid phylogeny, including the much contested monophyly of the Afro-Malagasy radiation, received strong support as well (localPP > 0.95). Based on their well resolved phylogenies, Articles **I** and **II** accorded familial status to *Micrelaps* and *Psammodynastes* as Micrelapidae and Psammodynastidae respectively (discussed in further detail in the Systematics section). While the phylogenies inferred in Articles **I** and **II** were consistent across methods, with strong branch support, those from the previous Sanger and small scale target capture loci-based studies (e.g., Vidal *et al.* 2008; Kelly *et al.* 2009; Pyron *et al.* 2011, 2013, 2014; Figueroa *et al.* 2016; Zaher *et al.* 2009, 2019; Zheng & Wiens, 2016; Weinell & Brown, 2018) did not show any significant degree of similarity to Articles **I** and **II** trees nor were they much congruent amongst themselves. The AHE phylogeny of Burbrink *et al.* (2020), however, was highly congruent with Articles **I** and **II** phylogenies. Cyclocoridae, *Psammodynastes* and Elapidae were the early diverging splits in their tree and the Afro-Malagasy radiation was monophyletic, with *Micrelaps* (regarded to be an atractaspidid by Burbrink *et al.*) as the basalmost Afro-Malagasy branch. Atractaspididae *sensu stricto* and Psammophiidae were sister clades. However, Burbrink *et al.*'s (2020) sampling did not include any *Buhoma*, Prosymnidae and Pseudaspididae.

Concatenated UCE data-based ML topologies in both Articles **I** and **II** and BI topology in Article **II** mostly matched that of the multispecies coalescent phylogenies (Figure 2 of Article **I** and **II**). The only consistent disagreement with coalescent trees was the relationships between lamprophiids, pseudaspidids and prosymnids. In the concatenation-based species trees, pseudaspidids were sister to Prosymnidae+Lamprophiidae. This relationship, however, did not receive strong SH-*alrt* and UFBoot in any of the concatenation trees. Three species of *Buhoma*, namely *B. depressiceps*, *B. marlieri* and *B. procterae*, were represented by traditional mitochondrial markers in the concatenated dataset of Article **II**. Concatenated UCE+traditional marker ML and BI topologies recovered *Buhoma* spp. as monophyletic and sister to Pseudaspididae. This clustering, however, did not receive statistical support.

The ASTRAL coalescent and ML concatenation phylogenies inferred in Article **III** based on a different, mixed set of target capture loci recovered a similar sequence of basal splits within Elapoidea, namely Cyclocoridae being the basalmost diverging branch followed by *Psammodynastes* and Elapidae (Figure 2 of Article **III**). The Afro-Malagasy radiation was again recovered as monophyletic within Micrelapidae representing the earliest diverging split. However, within the Afro-Malagasy group, inferred relationships differed somewhat from those estimated in Articles **I** and **II**. Atractaspididae and Psammophiidae were sister taxa. The ASTRAL phylogeny had *Buhoma marlieri* as the sister to Atractaspididae+Psammophiidae. Lamprophiidae, Pseudaspidae, Pseudoxyrhophiidae and Prosymnidae formed a polytomy in the ASTRAL species tree. In the ML tree, *Buhoma marlieri* was sister to Pseudoxyrhophiidae+Lamprophiidae. Prosymnidae and Pseudaspidae were sister to each other and this clade was sister to the clade comprised of *Buhoma*, Lamprophiidae and Pseudoxyrhophiidae. None of the phylogenetic relationships within the Afro-Malagasy radiation that differed from the ones in Articles **I** and **II** received significant LocalPP ( $\geq 0.95$ ) or UFBoot ( $\geq 95$ ) support.

Interestingly, analyses on the Article **IV** dataset, which was the most diverse and the biggest in terms of the variety and the number of sampled loci, yielded a somewhat different arrangement at the basal part of the elapoid phylogeny. Elapidae was the earliest branching clade in wASTRAL (hybrid and branch length weighting schemes; Figure 3; Figure 1 of Article **IV**), CASTER-Pair and CASTER-Site multispecies coalescent phylogenies (Supplementary figures of Article **IV**). Psammodynastidae was recovered as the sister taxon of Cyclocoridae. The Cyclocoridae+Psammodynastidae clade in turn was the sister to the Afro-Malagasy group. Phylogenetic placements of the family group taxa within the Afro-Malagasy clade in the wASTRAL species trees were identical to the coalescent topologies inferred in Articles **I** and **II**. The focal taxon of this study, *Buhoma* (represented by *B. marlieri*) was recovered as the sister of Atractaspididae with high LocalPP (0.97 in both weighting schemes) support. All the higher-level relationships, barring Prosymnidae+Pseudaspidae, were well supported (LocalPP > 0.95). The CASTER-Pair tree differed from wASTRAL tree in having Pseudaspidae as the sister to Lamprophiidae and Prosymnidae as a split basal to Lamprophiidae+Pseudaspidae. This tree also placed *Buhoma* as a basal split to (Prosymnidae (Lamprophiidae, Pseudaspidae)) clade with low LBBot (43.9) support. The LBBot of CASTER is more directly predictive of the estimated topological accuracy than commonly used branch support statistics like alrt and LocalPP (Zhang *et al.* 2025). Therefore, I regard the

position of *Buroma* in the CASTER-Pair tree as a potentially incorrect placement. Usually simulated branches receiving high LBBboot (equal or close to 100) are correctly placed whereas incorrectly placed branches receive low support. All the other deeper level relationships received > 90% LBBboot support in CASTER-Pair tree. CASTER-Site tree, however, had some unexpected phylogenetic placements. This tree had Micrelapidae and Pseudaspidae nested within Psammophiidae with poor LBBboot support. Recent phylogenomic assessments (Articles I - III) using different sets of markers and tree inference methods have been unequivocal about the placement of Micrelapidae as the basalmost split of the Afro-Malagasy radiation. The CASTER-Pair and wASTRAL topologies of the Article IV itself also placed *Micrelaps* as the sister to the rest of the Afro-Malagasy clade with high to the highest possible (LBBboot 92.1 and LocalPP 1) branch support. Pseudaspidae, consisting of *Pseudaspis cana* and *Pythonodipsas carinata*, also has an uncontroversial taxonomic status as a distinct subfamily/family (e.g., Kelly *et al.* 2009; Pyron *et al.* 2011, 2013, 2014; Zheng & Wiens, 2016; Figueroa *et al.* 2016; Articles I - III). Considering this and the low LBBboot values on these branches, I regard the poorly supported placement of Micrelapidae and Pseudaspidae within Psammophiidae by CASTER-Site as a methodological artefact. This implementation of CASTER uses HKY model which may be inadequate to handle some highly divergent loci. Despite being highly accurate, CASTER-Site has indeed been shown to be sensitive to high rates of mutation (Zhang *et al.*, 2025). Some other differences between CASTER-Site topology from those of CASTER-Pair and wASTRAL were – 1. The placement of prosymnids as a basal split to Atractaspidae, *Buroma*, Lamprophiidae, Psammophiidae (with nested Micrelapidae and Pseudaspidae), 2. The sister taxon relationship between Lamprophiidae and Psammophiidae and 3. No reciprocal monophyly between Aparallactinae and Atractaspinae. Interestingly these arrangements received LBBboot > 95. *Buroma marlieri* was the sister to Atractaspidae in the CASTER-Site tree with an LBBboot of 95.4, thus agreeing with the wASTRAL inference. Assuming poor LBBboot-supported branches as potential incorrect estimates, a 75% majority rule consensus on the deeper level elapoid relationships from two wASTRAL (hybrid and branch length-weighted) and the two CASTER (Pair and Site) species trees would therefore be – 1. Elapidae is the basalmost elapoid split, 2. Cyclocoridae+Psammodynastidae is the second most basal elapoid divergence and is sister to the Afro-Malagasy radiation, 3. The Afro-Malagasy radiation is monophyletic, Micrelapidae representing the earliest divergence within this clade, 4. Pseudoxyrhophiidae represents the second basalmost divergence within the Afro-Malagasy radiation, 5. *Buroma* is sister to Atractaspidae, 6. Aparallactinae and Atractaspinae are reciprocally monophyletic within

Atractaspididae, 7. Atractaspididae+*Buhome* and Psammophiidae are sister clades, 8. Lamprophiidae, Prosymnidae and Pseudaspidae is another clade sister to (Psammophiidae (Atractaspididae, *Buhome*)); however, resolution of the lamprophiid, prosymnid and pseudaspidae interrelationships is not unequivocal. Most of these fully or partly agrees with the previous, smaller (in terms of the number of elapoid taxa and loci) phylogenomic inferences (viz. Pyron *et al.* 2014; Burbrink *et al.* 2019; Articles I - III; Title *et al.* 2024).

Articles I, II and IV sampled multiple genera of each elapoid family/subfamily. Article IV had an especially dense sampling of genera. Unlike the higher-level relationships, the intergeneric relationships within elapoid families/subfamilies have not been highly controversial or unstable. Within Elapidae the Asian coral snakes of genus *Calliophis* have often been recovered as the earliest diverging group in previous molecular phylogenetic works (e.g., Pyron *et al.* 2013; Figueroa *et al.* 2016; Zaher *et al.* 2019). The multispecies coalescent topologies in Article IV recovered *Calliophis bivirgatus* to be the basalmost split within Elapidae. As can be seen later, recovery of Asian coral snakes at the base of Elapidae, itself representing the most basal clade within Elapoidea, has implications for elapoid biogeography. An interesting clustering in wASTRAL and CASTER-Pair topologies was *Hemibungarus mcclungi* and *Ophiophagus hannah*. Two previous molecular phylogenetic studies, namely Castoe *et al.* (2007) and Brown *et al.* (2018), also recovered this rather surprising relationship. A recent report by Brown *et al.* (2022) noted the threat display of Phillipines coral snakes of genus *Hemibungarus* with a narrow hood resembling that of King Cobras (*Ophiophagus* spp.). More interestingly, the basalmost split within King Cobras, that have recently been the subject of systematic revision (Shankar *et al.* 2021; Das *et al.* 2024), is the Phillipine King Cobra (*O. salvatana*). It therefore seems plausible that the early divergence between the common ancestral lineage of *Hemibungarus* spp. and *Ophiophagus* spp. took place in the Far East and common ancestry indeed explains the similar threat display behaviour in these two genera. For Afro-Asian, Australasian and marine elapid genera, the species trees in Article IV were mostly in agreement with the previous molecular phylogenetic works (e.g., Sanders & Lee 2008; Sanders *et al.* 2013; Pyron *et al.* 2013; Zaher *et al.* 2019; Maryan *et al.* 2023).

The phylogenetic relationships within Cyclocoridae in Article IV trees matched those in Weinell *et al.* (2020) except that *Levitonius* was recovered as the sister to *Myersophis* and not *Oxyrhabdium* as was the case in the Weinell *et al.* (2020)

Within the Afro-Malagasy radiation, Pseudoxyrhopiidae was the most densely sampled taxon in Article **IV**. This is also the most thoroughly studied (Ruane *et al.* 2015; Burbrink *et al.* 2019, 2023; DeBaun *et al.* 2024) Afro-Malagasy group family from a phylogenomic perspective. The phylogenies inferred by Burbrink *et al.* (2019) and DeBaun *et al.* (2024) had an especially dense sampling and Article **IV** topologies were congruent with them. However, the position of *Pseudoxyrhopus tritaeniatus* was not stable and neither wASTRAL nor CASTER clustered this species with the other two congeners. This is not unexpected given the data from *P. tritaeniatus* came from Article **III** whereas those for *P. ambreensis* and *P. microps* were from Title *et al.* (2024). The overlap between Article **III** MyBaits-20 custom probe set and the SqCL probe set of Title *et al.* is limited to a few UCE loci. This likely resulted in inadequate phylogenetic signal to correctly recover the monophyly of *Pseudoxyrhopus* spp.

Intergeneric relationships within Lamprophiidae in the target capture phylogenies from Articles **I**, **II** and **IV** showed certain consistent disagreements with traditional marker-based trees (e.g., Kelly *et al.* 2011; Pyron *et al.* 2011, 2013; Figueroa *et al.* 2016; Zaher *et al.* 2019; Tiutenko *et al.* 2022). Across datasets and methods, *Bothrophthalmus* has always been a highly supported basalmost lineage in the phylogenomic estimates as opposed to a more nested position in Sanger sequence-based topologies. *Lycophidion* has never been recovered as the basalmost split (as in Zaher *et al.* 2019) or as member of a subclade with *Hormonotus*, *Inyoka* (not sampled in Articles **I**, **II** and **IV**) and African file snakes of genera *Gonionotophis*, *Gracililima*, *Limaformosa* and *Mehelya* (as in Kelly *et al.* 2011; Pyron *et al.* 2011, 2013; Tiutenko *et al.* 2022). Rather *Lycophidion* were found to be the sister taxon of *Chamaelycus*, another poorly studied genus, in Article **IV** species trees (and to *Boaedon* in Articles **I** and **II** which did not sample *Chamaelycus*). This clustering in turn was sister to (*Bofa* (*Boaedon*, *Lamprophis*)).

For the sampled psammophiid genera, inferred relationships in Articles **I**, **II** and **IV** (wASTRAL and CASTER-Pair but not CASTER-Site) were mostly congruent with previous studies (e.g., Kelly *et al.* 2008; Figueroa *et al.* 2016; Zaher *et al.* 2019). As has already been discussed, the rather odd topology in this part of the CASTER-Site tree is probably attributable to methodological artefact.

An interesting case of disagreement amongst the species trees from Articles **I** and **II** versus those from Article **IV** concerns the relationships within Atractaspididae. *Homoroselaps* has historically been a source taxonomic debate (e.g., Bogert 1940; McCarthy 1985; McDowell

1986; Underwood & Kochva 1993; Cadle 1994). Early molecular phylogenetic works on snakes (e.g., Vidal & Hedges 2002; Lawson *et al.* 2005; Nagy *et al.* 2005) found *Homoroselaps* to be an atractaspidid, though whether it is sister to *Atractaspis* (e.g., in Vidal & Hedges 2002 and Nagy *et al.* 2005) or not (e.g. Lawson *et al.* 2005; Kelly *et al.* 2009) was unsettled. Subsequent studies (Pyron *et al.* 2011, 2013; Figueroa *et al.* 2016; Portillo *et al.* 2018, 2019; Zaher *et al.* 2019) with a dense taxon sampling (albeit with a sparse matrix) invariably recovered *Homoroselaps* as sister to *Atractaspis*. All the other atractaspidid genera have usually been found to form a subclade that is monophyletic with respect to *Atractaspis*+*Homoroselaps*; these genera have been allocated to Aparallactinae subfamily while *Atractaspis* and *Homoroselaps* have been placed in the Atractaspidinae in systematic revisions (e.g., Pyron *et al.* 2013; Portillo *et al.* 2018, 2019; Zaher *et al.* 2019). Surprisingly the UCE-based trees in Articles **I** and **II** could not recover reciprocally monophyletic Aparallactinae and Atractaspidinae. *Homoroselaps* was the earliest diverging lineage within Atractaspididae in those phylogenies and *Xenocalamus* was the sister taxon of *Atractaspis*. In contrast, the wASTRAL and CASTER-Pair trees (but not CASTER-Site) recovered *Homoroselaps* clustered with *Atractaspis* with strong LocalPP and LBBoot supports respectively. The genera traditionally assigned to Aparallactinae were also monophyletic in wASTRAL and CASTER-Pair trees. However, except addition of more species of genera *Aparallactus* and *Atractaspis*, the taxon sampling in Article **IV** does not differ much from that in Articles **I** and **II**. The AHE phylogeny of Burbrink *et al.* (2020), which included *Aparallactus*, *Atractaspis* and *Homoroselaps*, also recovered a *Atractaspis*+*Homoroselaps* cluster. Therefore, it seems that a larger and more diverse array of target capture markers in Article **IV**, as opposed to only UCes used in Articles **I** and **II**, allowed for recovery of Aparallactinae and Atractaspidinae. Nevertheless, it noteworthy that Atractaspididae itself also represents a rapid radiation (Portillo *et al.* 2018, 2019; Article **I**), with very short deep branches, a hypothesis of polytomy for this part of the tree could not be rejected (Article **I**) and CASTER-Site tree of Article **IV** recovered *Homoroselaps* as sister to *Polemon*. Hence, although Article **IV** advocates for subdivision of Atractaspididae within Aparallactinae and Atractaspidinae, it should be noted that intergeneric relationships within this family do have an element of uncertainty.

The traditional mitochondrial, nuclear and mito-nuclear phylogenies estimated in Article **II** showed marked incongruence amongst themselves and with the UCE topologies and had *general* low branch support at the deeper level. Concatenation of mitochondrial markers with UCes did not change the topology but lowered UFBoot support throughout the tree. Fast

evolving mitochondrial sequences seems to be especially unsuitable for the study of rapid radiations in the deep past. Probably by exacerbating the problem of obfuscation of the already low phylogenetic signal on the short branches with a high degree of mutational saturation, mitochondrial markers seem to have a negative impact on the tree inference in this case (Article II). Also, the poor performance of limited number of traditional markers has been a common theme in elapoid phylogenomics. It therefore appears that loci-sparse datasets, especially traditional mitochondrial markers, are mostly inadequate for resolving old, rapid diversifications like Elapoidea.

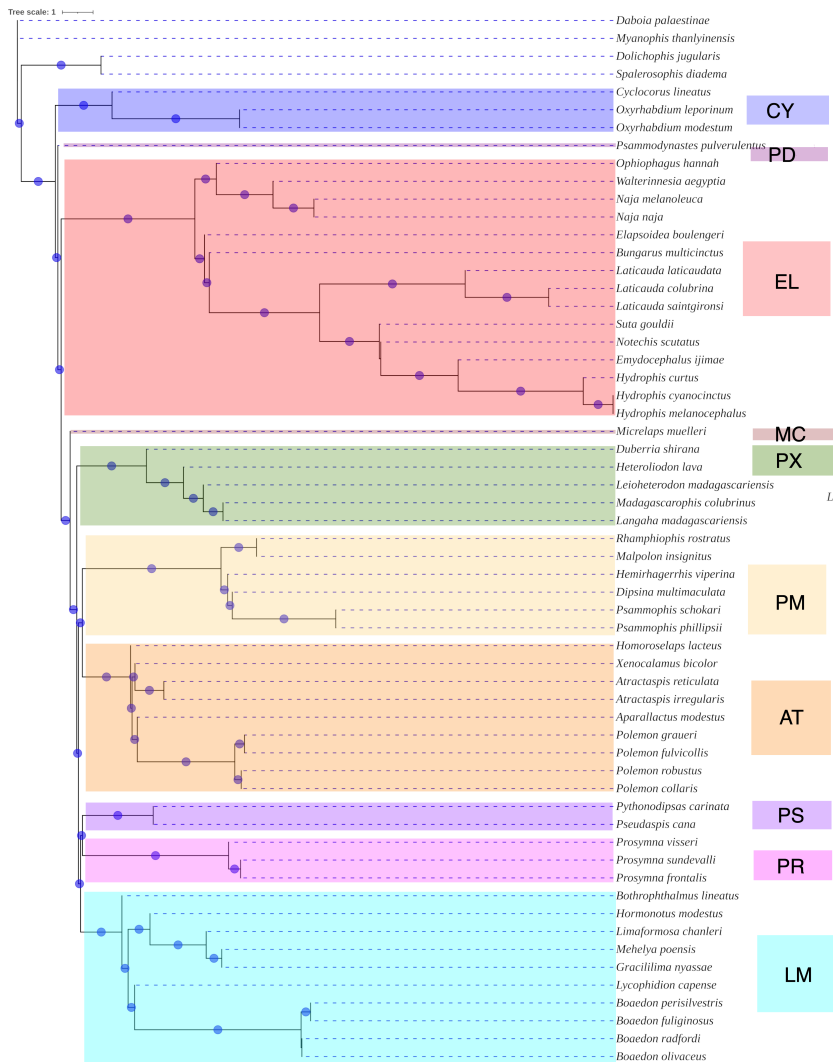


Figure 2: wASTRAL-hybrid multispecies coalescent phylogeny from Article II based on ultraconserved elements. AT – Atractaspidae, CY – Cyclocoridae, EL – Elapidae, LM – Lamprohiidae, MC – Micrelapidae, PM – Psammophiidae, PR – Prosymnidae, PS – Pseudaspidae, PX – Pseudoxyrhophiidae.

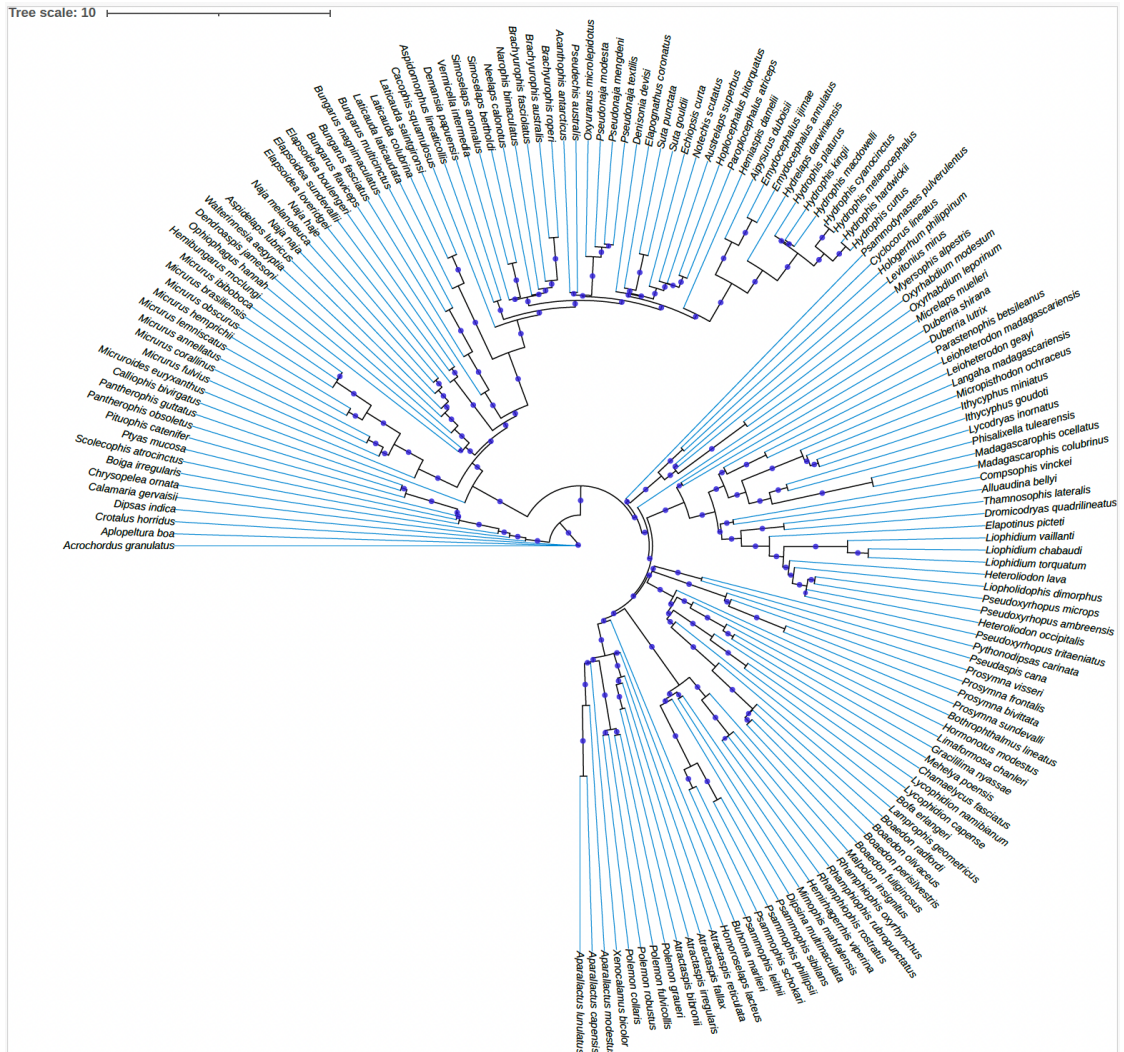


Figure 3: wASTRAL-hybrid multispecies coalescent phylogeny from Article IV based on ultraconserved elements, anchored hybrid enrichment loci, rapidly evolving exons, ddRAD-like loci and trait-associated loci. Blue circled on the branches indicates > 0.95 LocalPP support.

## 4.2. A study of potential confounding factors

Several factors can make an ancient, rapid radiation difficult to resolve. ILS on the very short, deep branches is a well-recognised confounding factor (e.g., Whitfield & Lockhart 2007; Whitfield & Kjer 2008; L veill -Bourret *et al.* 2018; Cloutier *et al.* 209). Other confounding factors that often accompany rapid diversification in introgression and hybridisation (e.g., Vanderpool *et al.* 2020; Esquerr  *et al.* 2022).

However, a factor much simpler than those biological causes, that is sequence alignment and/or gene tree estimation error, can also lead to spurious phylogenies (Comte *et al.* 2023; Hutter & Duellman 2023). To rule out that this is the case, Article **IV** filtered outlier genes (i.e., outlier species in individual locus tree) with PhylteR. None of the loci and species were identified as total outliers. A wASTRAL (branch length weighted) analysis on a filtered gene tree set (i.e., branches in individual gene trees detected as outliers by PhylteR discarded) returned a phylogeny identical to the wASTRAL trees inferred from unfiltered gene trees.

Article **I** tested for the presence of true multifurcation events in the elapoid tree. However, at  $\alpha = 0.05$ , the hypothesis of hard polytomy in the ASTRAL species tree were rejected. At an  $\alpha = 0.01$ , all the hypotheses of hard polytomy, except that involving *Homoroselaps*, could still be rejected. It therefore appears that there are no true multifurcations in the phylogeny of Elapoidea.

Articles **I** - **III** tested for incomplete lineage sorting using gene and site concordance and discordance factors to test for ILS (Figure 2 of Article **III**). The gCF and sCF factors tended to be low for the deep branches within the Afro-Malagasy radiation (Articles **I** - **II**). The sCF was around 33% on several, though not all, short ‘rapid radiation phase’ branches, indicative of probably both a paucity of decisive phylogenetic signal and ILS for these splits. Article **III** explored concordance and discordance factors more elaborately. The gCF values were greater than gDF1 and gDF2 at most nodes in Article **III** which is suggestive of relatively low gene tree-species tree discordance. The sDF1 and sDF2 on ASTRAL and ML trees showed significant difference in only 0.05% of  $\chi^2$ -tests and the no node had a significant sDF1 and sDF2 difference in more than one bootstrap. This is indicative of a primarily ILS-driven discordance pattern.

Results of multispecies network coalescent analyses from Articles **II** and **III** to detect potential gene flow events had disagreements. The PhyloNetworks analyses with 2, 5 and 6 permissible reticulations in Article **II** had the best log-likelihoods (Figure 3 of Article **II**). None

of these networks had more than two inferred reticulation events of which none achieved an inheritance probability close to 0.5. PhyloNet, on the other hand, yielded more horizontal edges with the increasing number of permissible reticulations. PhyloNet networks with 4, 5 and 6 reticulations received the best log-likelihood values. A reticulation event involving Lamprophiidae (or an extinct/unsampled sister lineage) and Atractaspididae, with minor edge inheritance probability ranging from  $\sim 0.25$  (in PhyloNetworks) to approaching 0.5 (in PhyloNet), was a consistent feature in all of these inferred networks. From a biogeographic point of view, gene flow between ancestral lineages leading to modern atractaspidids and lamprophiids is not improbable. However, the 0.5 inheritance probability should not be interpreted as the evidence of a hybrid origin as – 1. continuous migration can produce such a pattern (Tiley *et al.* 2023), 2. multispecies coalescent networks cannot distinguish between different types of reticulations (Cao *et al.* 2019) and 3. PhyloNetworks and PhyloNet disagreed over the inheritance probability for this horizontal edge.

Reticulations involving Cyclocoridae, Elapidae and Psammodynastidae were also inferred in number of networks. Given that the early divergence of elapoids possibly took place in Asia, such gene flow events are also not totally implausible. Furthermore, both PhyloNetworks and PhyloNet inferred certain reticulation edges that do not originate from a sampled lineage. Such edges have been interpreted as ghost introgression, i.e., a gene flow event from an unsampled or extinct lineage (e.g., Árnason & Halldórsdóttir 2019). This, however, has been shown to be problematic as only full likelihood/Bayesian implementations, and not MPL ones used in Article **II**, of multispecies coalescent networks can distinguish ghost introgressions (Pang & Zhang 2024). However, ghost introgression is not unlikely given that Elapoidea is an old radiation and many early lineages that could have contributed genetic material via introgression are possibly extinct (Ottenburghs 2020). However, if this has indeed been the case, it calls for even more caution in interpreting the results of MPL networks as ghost introgression has been demonstrated to significantly mislead commonly used gene flow detection methods (Tricou *et al.* 2022; Pang & Zhang 2024).

In the contrary, the best PhyloNet MPL topology selected under the Djump algorithm in Article **III** did not have any reticulations.

Article **IV** uses a different, recently developed approach implemented in PhytoP to look for potential signatures of ILS and/or gene flow (Figure 2 of Article **IV**). All the deeper level relationships except the basalmost split of Elapidae showed strong signal of ILS, mostly with

ILS-i > 70%. The signal of introgression, IH-i, was not higher than ILS-i on any splits though there were strong introgression signals on several deep branches. There were strong (IH-i 42.7% and  $q_2 > q_3$ ) signal of introgression from *Buhome* and probably the stem psammophiid to atractaspids. The ILS-i on this topology was 60.6%, much lower the value observed on several other parts of three. It thus seems ancient introgression may explain underlying conflict in this part of the elapoid tree. The relationship between prosymnids and pseudaspids bears signature of both strong ILS (ILS-i 93.7%) and introgression (IH-i 42.75). The sister relationship between Lamprophiidae and (Prosymnidae, Pseudaspidae) also has an ILS-i of 86.4% and a relatively low IH-i of 19.9%. However,  $q_2$  is not much higher than  $q_3$  in either of these and therefore, strong ILS probably is a better explanation behind the difficulties experienced in resolving this part of the Afro-Malagasy radiation. There are also prominent signatures of both ILS (ILS-i 70.5%) and introgression (IH-i 38.6% and  $q_2 > q_3$ ) involving probably the ancestral lineages of Cyclocoridae, Elapidae, Psammodynastidae and the Afro-Malagasy group.

It seems alignment or gene tree estimation error and outlier genes/taxa is not much of an issue with the elapoid target capture datasets. Elapoidea also do not have any truly multifurcating deep nodes. The signature of ILS, however, is very prominent on short branches and there is broad agreement on this between different approaches adopted in Articles **I - IV**. Ancient gene flow is a more contentious issue. Introgression may explain the underlying conflict to some extent on some branches. However, ILS and low phylogenetic signal on the short, 'rapid radiation phase' branches seem to be the primary confounding factors, with a probable, relatively minor contribution from past gene flow.

### **4.3. Divergence time**

Divergence time estimates in Articles **I, II** and **IV** were similar (Figure 3A and B; Figure 3 of Article **I**, Figure 4 of Article **II**, Figure 3 of Article **IV**) and are generally older than those in Article **III** (Figure 3 of Article **III**). All the time-trees from Articles **I, II** and **IV** placed the origin of Elapoidea from 50.2 – 54.6 MYA in the early Eocene. The early divergence into the ancestral lineages of all the family level elapoid clades were estimated to have taken place between ~54 MYA and ~40 MYA in the early to middle Eocene in the site-based time-trees. The branch length-based Timetree from Article **IV** estimated comparatively younger ages, placing the divergence between Cyclocoridae and Psammodynastidae at 36.5 MYA and those between Afro-Malagasy families from 40.4 MYA to 32.3 MYA in the middle to late Eocene





been recovered as the basalmost branch within Elapidae. The implication is that even if Elapidae, with many taxa in Asia, Africa, Australasia and the Americas, is the basalmost clade instead of the exclusively Asian cyclocorids, an ‘Out of Africa’ scenario would still be unlikely.

Article **III**, which specifically focused on testing this biogeographic hypothesis, inferred a phylogeny congruent to those from Articles **I** - **II**, with cyclocorids and psammodynastids representing the oldest diverging lineages. Expectedly, the most optimal biogeographic model, BAYAREALIKE+J, of Article **III** reconstructed an Asian origin for Elapoidea, with multiple ‘Out of Asia’ colonisations of Africa (Figure 4 of Article **III**). These colonisations involved – 1. The common ancestor of the Afro-Malagasy clade in the late Eocene and Oligocene, 2. The common ancestor of African and middle eastern cobras, namely *Aspidelaps*, *Hemachatus*, *Naja*, *Pseudohaje* and *Walterinnesia* in the early to middle Miocene, 3. The common ancestor of African garter snakes (*Elapsoidea* spp.) in late Oligocene and 4. The common ancestor of mambas (*Dendroaspis* spp.) in the early Miocene. Amongst these, the Montpellier and Moila snakes (*Malpolon* spp.) colonised Europe and sand snakes (*Psammophis* spp.) and true cobras (*Naja* spp.) colonised back into Asia in the early Miocene. Apart from these, elapoids have also colonised from Asia the Americas, Australasia and from Australasia, the marine habitats.

Somewhat different phylogenetic relationships inferred in the Article **IV** necessitated reanalysing the biogeographic hypotheses. Article **IV** had a particular dense taxon sampling for Elapoidea. All the species trees in this Article inferred Elapidae as the earliest diverging clade within Elapoidea, with *Calliophis* being the basalmost elapid split. As postulated in Articles **I** and **III**, the best biogeographic model, DEC+J, still favoured an Asian origin of Elapoidea for this topology (Figure 5; Figure 4 of Article **IV**). The DEC+J reconstruction supported three ‘Out of Asia’ colonisation events into Africa, namely by – 1. The common ancestor of the Afro-Malagasy clade in the middle Eocene, 2. The common ancestor of cobras and mambas in the late Oligocene and 3. The common ancestor of African garter snakes in the late Oligocene. The DEC+J also fully corroborated the findings of Article **III** on the colonisation of the Americas, Australasia and the marine realm and colonisation of Europe and recolonisation of Asia.

An ‘Out of Africa’ biogeographic hypothesis, favoured for Elapoidea by Kelly *et al.* (2009), has been proposed for several other vertebrate groups as well (e.g., Sen 2013; Pyron *et al.* 2014; Alencar *et al.* 2016; Weil *et al.* 2022). However, the elapid phylogenies inferred from target capture data consistently recovered either elapids, with Asian *Calliophis* as their basalmost

split, or exclusively Asian cyclocorids as the earliest diverging branches of Elapoidea. This already makes an African origin highly unparsimonious. The most optimal biogeographic reconstruction models in Articles **III** - **IV** clearly supported an Asian origin, followed by multiple bouts of colonisation of Africa and the other continents. This allows for definitive rejection of an ‘Out of Africa’ scenario for the early elapoids.

Limenitidine butterflies are an example of a group for which an early Eocene origin in Asia, followed by ‘Out of Asia’ dispersal into Africa by the late Eocene has been proposed (Tseng *et al.* 2022). Interestingly, this butterfly radiation also has instances of Oligocene and Miocene back-and-forth colonisation and recolonisation of Asia and Africa just like the elapoids. Moreover, limenitidines also colonised the Americas and the Australasia from Asia. The estimated time of origin, which possibly took place in East Asia, of limenitidines is ~52 MYA, coinciding with the time of elapoid origin (Articles **I**, **II**, **IV**). This interesting parallel with elapoid diversification and biogeographic history seems to indicate the Eocene ‘Out of Asia’ scenarios might be more common than usually appreciated.

The first colonisation of Africa by the ancestor of the Afro-Malagasy clade likely was a trans-Tethys dispersal event, a mechanism proposed for other groups of vertebrates as well (e.g., Beard 2016). Assuming the somewhat older divergence time estimates from Articles **I**, **II** and **IV** to be correct, migration across the Tethyan seaway seems more plausible for two subsequent, late Oligocene rounds of colonisation of Africa by elapids than over the *Gomphotherium* land bridge (Harzhauser *et al.* 2007) which existed in the early to middle Miocene. However, this land bridge was probably the route to recolonisation of Asia by cobras and sand snakes.

Colonisation of Australasia from Asia by elapids took place around the middle Miocene, possibly by island-hopping trans-oceanic dispersal. Such Miocene faunal and floral exchange between Asia and Australasia has been proposed for other groups as well (e.g., Gauffre-Autelin *et al.* 2021; Peng *et al.* 2021). Increasing forest cover in the intervening chain of islands in the Miocene (Kooyman *et al.* 2019; Peng *et al.* 2021) and ecological opportunities in Australia perhaps facilitated and promoted a southward migration. Upon reaching Australasia, elapids experienced a rapid radiation, including two instances of land-to-marine transition (Sanders & Lee 2008; Article **IV**).

The colonisation from Asia into the Americas by the ancestor of American coral snakes (*Micrurus*, *Micruroides* and *Leptomicrurus*) might have happened via the Beringia land



#### 4.5. Cranial osteology

Articles **I**, **II**, **IV** and **V** studied a vast number of  $\mu$ -CT scans of the crania of elapoid snakes. Primary objectives of the comparative cranial osteology were finding diagnostic features for the newly described families. Another motivation for this work was to aid determination of the phylogenetic affinities of genera lacking any molecular data.

Article **I** described family Micrelapidae to which *Micrelaps* spp. and *Brachyophis revoili* were assigned. Of these, *Brachyophis revoili* is a very rare monotypic snake for which no suitable tissue sample exists. The shaft of the ectopterygoid in both genera is conspicuously expanded at the point of articulation with the pterygoid (Figure 4 of Article **I**). The lateral expansion projects out as a posterolaterally directed protuberance. This character was interpreted as a synapomorphy diagnosing Micrelapidae as a family consisting of the type genus *Micrelaps* and monotypic *Brachyophis*. This ridge possibly serves as an attachment site for the pterygomandibularis muscle (Das & Pramanick 2019).

Article **II** described Psammodynastidae to accommodate *Psammodynastes* spp. Maxilla of *Psammodynastes* possesses two small, ungrooved teeth at the rostral end, followed by 1–2 enlarged, ungrooved teeth, next 5–9 small, ungrooved teeth and at the caudal end, and two enlarged, grooved fangs (Figure 5 of Article **II**). This maxillary dentition is very similar that of *Psammophis* but the lack of a prominent canthal ridge and moderate sized optic foramen in *Psammodynastes* (vs. prominent canthal ridge and a large, lizard-like optic vacuity in *Psammophis*) diagnoses it from the former. The parietal of *Psammodynastes* also bears an unusually large, squarish postorbital crest. Among the examined taxa, only *Ophiophagus* and *Notechis* have a postorbital crest this well-developed but a maxilla with front fangs in these elapids easily distinguishes them from the rear-fanged *Psammodynastes*.

Article **IV** proposed for *Buhoma* spp. its own family. Unlike micrelapids and *Psammodynastes*, *Buhoma* spp. do not have any synapomorphy that can facilitate unequivocal diagnosis from all the other elapoids (Figure 6; Figure 5 of Articles **II** and **IV**). However, a combination of characters, namely – 1. Prominent perforation in the prefrontal lateral lamina, 2. Maxillary dentition comprising of 16–19 equal sized, ungrooved, small teeth and two enlarged, grooved rear fangs, 3. Lack of a caudally extended dentigerous ‘tail’ from the maxillary ectopterygoid process, 4. Lack of any fossorial or arboreal adaptation in the snout complex bones, 5. Lack of canthal ridge, 6. Moderate sized optic foramen, 7. Ectopterygoid

shaft devoid of lateral protuberance, 8. A prokinetic joint formed by the septomaxillae and the frontal medial pillars, helps to distinguish *Buhoma* spp. from all the examined elapoid genera.

Article V investigated the phylogenetic relations of *Hypoptophis wilsonii*, a poorly known central African atractaspidid (Figure 1-23 of Article V). BI and MP phylogenies, inferred with a matrix of 61 cranial osteological characters, recovered *Hypoptophis* as sister to *Aparallactus*, albeit with poor branch support. The phylogenies recovered Atractaspidinae, consisting of *Atractaspis* and *Homoroselaps*, as monophyletic though genera usually assigned to Aparallactinae were not monophyletic. Assuming the clustering of *Aparallactus* and *Hypoptophis* to be correct and following the classification scheme adopted in Article IV, *Hypoptophis* can be regarded as a member of the Aparallactinae subfamily.

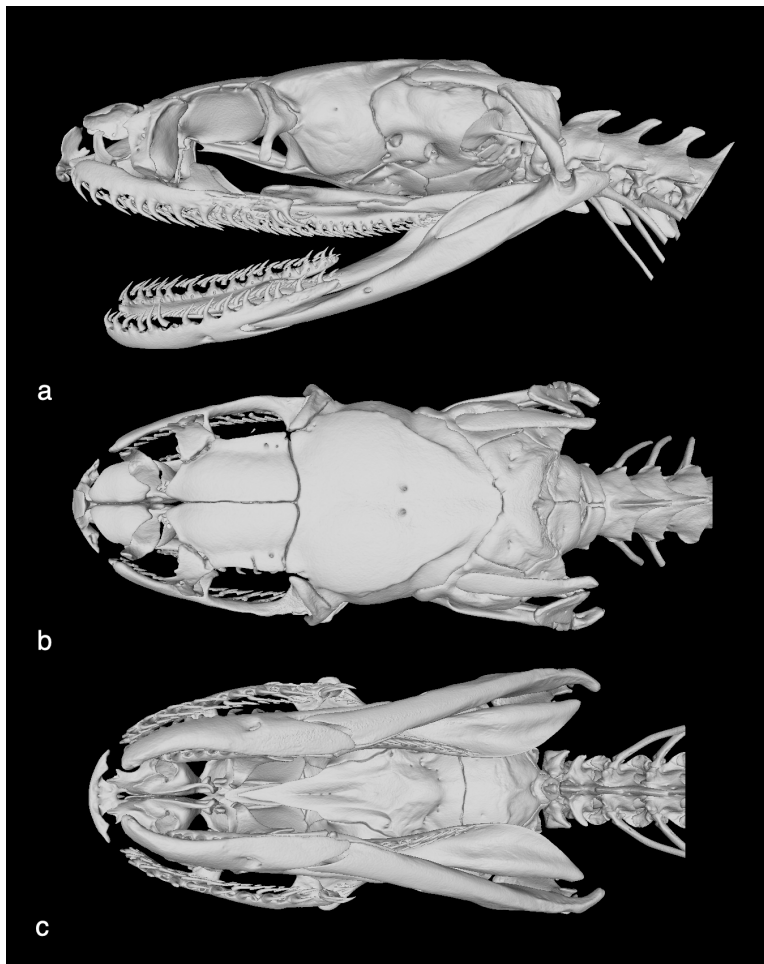


Figure 6. Cranium of the holotype of *Buhome marlieri* (Royal Museum of Central Africa – BE-RMCA-VER-REP 18091).

#### 4.6. Systematics

Stabilising the higher-level classification of Elapoidea was the primary objective of this doctoral dissertation. Inference of well-supported phylogenies with mostly consistent and congruent topologies and thorough study of cranial osteology allowed for realisation of that goal. Articles **I** and **II** classified all the Afro-Malagasy clades except the micrelapids as subfamilies under Lamprophiidae. The monophyly of the Afro-Malagasy radiation has invariably been recovered in all the phylogenomic studies of this thesis and therefore, the classification scheme adopted in Articles **I** and **II** is congruent with the phylogeny. However, it seems this classification has not been widely adopted, with databases (e.g., Reptile Database [Uetz *et al.* 2025]) and taxonomic and phylogenomic literature (e.g., Roberts *et al.* 2024) still predominantly treating Afro-Malagasy clades as families. Also, the exclusion of micrelapids from the family intended for the Afro-Malagasy clade, as was done in Articles **I** and **II**, can be considered rather arbitrary. Moreover, Article **IV** recovered aparallactines and atractaspidines as reciprocally monophyletic. Treating them as subfamilies of Atractaspididae better reflects the phylogenetic relationships of these semi-venomous and highly venomous (only *Atractaspis* spp.) snakes rather than treating both as subfamilies of Lamprophiidae. Therefore, Article **IV** adopted a classification where each major monophyletic group within the Afro-Malagasy radiation is treated as full family. Article **I** described family Micrelapidae to accommodate *Brachyophis* and *Micrelaps*. Article **II** described family Psammodynastidae for *Psammodynastes*. Finally, Article **IV**, which is not yet published, proposes allocating *Buhome* to its own family. To avoid potential confusion over the date of publication of the *Buhome* family group taxon nomen, I refrain from mentioning the name in the thesis synopsis. All these newly established families are well supported in the inferred phylogenies and are not nested within any recognised families. Therefore, description of them as new families is taxonomically justified. To the best of my knowledge, all known elapoid genera have now been assigned to a family group taxon.

To conclude, the classification scheme proposed in this thesis for snake superfamily Elapoidea assigns elapoid genera into eleven families, namely Atractaspididae (subfamilies Aparallactinae and Atractaspidinae), Cyclocoridae, ‘*Buhome* family’, Elapidae,

Lamprophiidae, Micrelapidae, Prosymnidae, Psammodynastidae, Psammophiidae, Pseudaspidae and Pseudoxyrhopiidae.

## 5. Conclusions and outlook

Reconstructing the tree of life of Earth's biodiversity is one of principal goals of biological sciences. Phylogenies serve as the cornerstone of systematics and comparative biology. Thanks to the advancement in molecular and computational biology, the snake tree of life is now mostly well resolved. However, one part of the snake phylogeny, superfamily Elapoidea, proved to be a recalcitrant challenge for the evolutionary biologist. This thesis is an endeavour to fill this last remaining major knowledge gap in the evolutionary tree of living snakes.

Article **I** was the first step – for the first time, a fully resolved phylogeny of Elapoidea, with strong statistical support, was inferred and showed remarkable stability across inference strategies. Genus *Micrelaps* was found to constitute a unique branch in this phylogeny and to accommodate this and *Brachyophis*, a new family Micrelapidae was established. Article **II** inferred the phylogenetic position of *Psammodynastes*, a viper-mimic. This genus too was found to represent an early diverging, unique branch in the elapoid phylogeny and was assigned to its own new family, Psammodynastidae. Articles **III** and **IV** looked into the biogeographic history of Elapoidea. The analyses conclusively supported an Asian origin followed by 'Out of Asia' dispersal into Africa. The early diversification of Elapoidea took place rapidly, possibly prompted by ecological opportunities, and all the major elapoid clades likely appeared in the Eocene. Article **IV** also resolved the position of genus *Buhoma* in the elapoid phylogeny and proposed a new family for this genus. The findings of the four articles suggest that incomplete lineage sorting has been main factor that made the inference of elapoid phylogeny so difficult, with low phylogenetic signal and possibly early introgression being additional confounding factors. Last but not the least, thorough cranial anatomical comparisons conducted in Articles **I**, **II**, **IV** and **V** provided diagnostic characters for the newly described families and helped to classify rare genera for which molecular data was not available.

The studies conducted for this thesis demonstrates that a diverse genome-wide dataset and state-of-the-art phylogenomic tools that can take confounding factors, such as incomplete lineage sorting and gene flow, into account can confidently resolve ancient rapid radiations. Researchers investigating such evolutionary scenarios will very likely benefit from leveraging genome scale data and robust multispecies coalescent tree and network estimation methods.

The nature of science is to always march ahead and this thesis too is not a tombstone but rather a building block. I envision researchers now armed with a robust phylogeny will find out exactly what palaeoecological and/or palaeoclimatic factors prompted the explosive

diversification of elapoid snakes. Two of the four families of medically significant venomous snakes are in the Elapoidea. I believe resolution of elapoid phylogeny will finally give us a complete picture on how snakes acquired such a diverse array of venoms and fangs. We are in a mass extinction-scale biodiversity crisis. I hope a stable taxonomy of a major group of snakes will allow conservationists, ecologists and policymakers to focus on combating the loss of biodiversity without finding themselves in an unfamiliar nomenclatural quagmire.

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## **Selected media coverages on the articles of the thesis**

1. Nature (2024) – ‘Cobras and mambas and coral snakes, oh my! DNA shows their origins’
2. Nature (India, 2024) – ‘Cobras originated in Asia and migrated to Africa’
3. Newsweek (2024) – ‘Snake Evolutionary Mystery Solved in 'Lifetime Achievement'’
4. LiveScience (2024) – ‘Evolution of snakes takes surprise twist — cobras didn't come from where we thought they did’
5. YLE (2024) – ‘Kolkatan käärmemies Helsingissä’
6. University of Helsinki press release (2024) – ‘Viper-mimicking snake from Asia is a unique branch in the reptile evolutionary tree’
7. Phys (2023) – ‘Family tree of major snake group rewritten and new branch of snakes found’
8. The Print (2023) – ‘New snake family–Micrelapidae–discovered, three species identified’
9. The Brussels Times (2023) – ‘Researchers discover new family of snakes’
10. Royal Belgian Institute of Natural Sciences press release (2023) – ‘Scientists discover new family of snakes’
11. Tel Aviv University press release (2023) – ‘New snake family identified’
12. The Jerusalem Post (2023) – ‘Israeli zoologists find new family of snakes dating back 50 million years’
13. Haaretz (2023) – ‘New snake family identified in Israel and Africa’
14. Ministry of Foreign Affairs, Israel (2023) – ‘Una nueva familia de serpientes – Micrelapidae’