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Moving Beyond Morphology: Genomic Insights Into Evolutionary Histories of Haplosclerid Sponges

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ABSTRACT

Accurate taxonomic identification is essential for defining species boundaries and understanding biodiversity. However, this remains challenging for groups where morphological character evolution is poorly understood or diagnostic traits are absent. Sponges (Phylum Porifera) exemplify this complexity due to their morphological simplicity and phenotypic plasticity. This study applies target capture enrichment of Clade-Specific Elements (CSEs), which target taxon-specific exon loci, to analyse genomic data from 181 specimens spanning five major families and 22 of the 28 recognised genera within the demosponge order Haplosclerida. Our dataset includes 105 type specimens, 42 of which are holotypes, historical material from renowned expeditions such as the HMS Challenger, and specimens dating back to 1795. To our knowledge, this is the first phylogenomic study of its kind based on a broad sampling of historical collection material of a species-rich sessile marine invertebrate taxon. Our analysis reveals pervasive non-monophyly across most internal groups and significant inconsistencies in morphology-based classification schemes. Convergent evolution and homoplasy obscure morphological characteristics, often leading to ambiguous taxonomic assignments and impeding our understanding of evolutionary relationships. This emphasises the urgent need for a genomic framework. Our type-focused phylogenomic approach tests evolutionary hypotheses and has the potential to facilitate robust taxonomic revisions. We argue that continued efforts on subjective morphological criteria are untenable and advocate for integrating genotypic data as the primary basis for species classification in Haplosclerida. Using genomic insights, this study contributes to an improved understanding of sponge biodiversity and evolutionary history and provides an objective foundation for testing species concepts.

1 | Introduction

Species identification is imperative for meaningful biological observations and reliable biodiversity assessments, and among the most fundamental questions in this context are 'What is a species?' and 'How many species are there?' (de Queiroz 2007; Adams et al. 2014). Since the introduction of the Linnaean classification system, which largely defined the hierarchical organisation of organismal groups according to their morphology, significant progress has been made in addressing the

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questions above across the various branches of the Tree of Life. Morphological-based taxonomy has been the subject of ongoing debate and refinement for centuries, but it has undeniably laid the very foundation for formalised taxonomy as we know it today and remains essential for understanding biodiversity patterns in many organisms. This is particularly true for groups where molecular approaches are of limited use, such as fossils and preserved specimens (Padial et al. 2010). Nevertheless, achieving unambiguous species identification remains a difficult task, partly because certain organisms are hard to study within their natural environment, their microscopic body size, or the high levels of cryptic or phenotypic diversity present (Sigwart and Garbett 2018). Also, recent advances in gene sequencing have challenged traditional morphology-based classifications by revealing previously underappreciated species diversity, and by providing new insights into character evolution in various groups of organisms (e.g., Fouquet et al. 2007; Lumbsch and Leavitt 2011; Morrow et al. 2019). While these insights are often based on single or few gene markers, such single-locus data can be limiting because they may not capture the full genetic complexity of an organism (Fujita et al. 2012). This limitation can result in difficulties detecting convergent evolution, where different species independently evolve similar traits, and in accurately resolving gene tree–species tree discordance, where the evolutionary history of a particular gene differs from the species' evolutionary history due to events such as incomplete lineage sorting or horizontal gene transfer (Will and Rubinoff 2004; Dupuis et al. 2012; Fujisawa and Barraclough 2013). Consequently, relying solely on single-locus data may lead to an incomplete or misleading understanding of evolutionary relationships. In addition, the effectiveness of specific markers for revealing species diversity varies across metazoan groups because these markers evolve at different rates in different lineages. Fast-evolving markers are suitable for distinguishing closely related species but may saturate too quickly to help resolve deeper nodes. Slow-evolving markers, on the other hand, might not provide enough resolution to distinguish between recently diverged species. The choice of marker must, therefore, be carefully considered in the context of the evolutionary rate of the group being studied, as using an inappropriate marker can lead to either under- or overestimation of species diversity (e.g., Shearer et al. 2002; Erpenbeck et al. 2005; Huang et al. 2008; Shearer and Coffroth 2008).

Thanks to the rapid advancements in high-throughput sequencing (HTS) technologies over the past decades, it is now possible to retrieve hundreds to thousands of loci across genomes (McCormack et al. 2013). This genome-wide data holds significant potential to overcome the challenges associated with single-gene marker approaches (Thomson et al. 2010). Target enrichment, or sequence capture, is a cost-effective method that allows for the selective capture of genome-wide loci from multiple DNA samples before sequencing (Mamanova et al. 2010; Jones and Good 2016). This technique shows great promise for taxonomic identification and biodiversity assessments (McCormack et al. 2013). Specifically, the target capture enrichment of ultraconserved elements (UCEs) has successfully addressed long-standing taxonomic questions, particularly in groups with molecular-morphological conflicts, such as corals (Quattrini et al. 2018; Bridge et al. 2023; Capel et al. 2024). UCEs, characterised by conserved regions across species flanked by highly divergent, phylogenetically informative regions, provide non-anonymous markers that have been demonstrated to enhance taxonomic precision (Faircloth et al. 2012). An additional advantage of this approach is its efficacy in collecting genomic data from specimens that contain short and degraded DNA fragments, as is often the case with (older) type material from natural history collections (McCormack et al. 2016; Derkarabetian et al. 2019; Rancilhac et al. 2020; Hahn et al. 2022; Connelly et al. 2024). Furthermore, target capture ensures effective contamination control, as the custom-made gene markers are of known homology (McCormack et al. 2017). Post-capture filtering removes off-target sequences that may arise from 'by-chance' similarity to other regions. It sets a minimum sequence identity threshold or filters out reads that map to multiple regions (Faircloth 2016). Collecting genomic data from natural history collections containing numerous type specimens can increase our understanding of evolutionary histories and identify cryptic species complexes (McCormack et al. 2017; Derkarabetian et al. 2019), but it is also valuable to re-evaluate current classification systems of taxonomically complex taxa.

Sponges (Porifera) are an example of a taxonomically complex group, with many unresolved internal classifications due to their simple bauplan and limited, often plastic morphological characters (Maldonado et al. 1999; Erpenbeck et al. 2006; van Soest et al. 2012), and varying evolutionary rates in most commonly used gene markers (Shearer et al. 2002; Erpenbeck et al. 2004, 2005). However, understanding their ancestral morphological and developmental character states is crucial for clarifying interrelationships, testing species boundaries, and gaining insights into early animal evolution (Nichols 2005; Wörheide and Erpenbeck 2007). Within this context, the demosponge order Haplosclerida presents by far, the biggest enigma in sponge systematics. This taxon comprises numerous diverse and evolutionary ancient lineages (McCormack et al. 2002) and is widely distributed across marine habitats but is especially known to be species-rich in tropical reef systems (Erpenbeck, Voigt, et al. 2016; Carballo et al. 2019). Internal relationships within Haplosclerida are difficult to resolve using the current suite of diagnostic characteristics, which typically include skeletal architecture, spicule types and dimensions, external morphology, and spongin fibres (see de Weerdt and van Soest 1986). These morphological traits are difficult to identify and assess reliably, as they can be highly plastic and sensitive to environmental conditions (e.g., Maldonado et al. 1999; Cárdenas and Rapp 2013), and frequently overlap between different and sometimes distant taxonomic groups (Diaz et al. 2007; Leal et al. 2023). As a result, traditional morphological methods alone, were soon regarded as insufficient for accurate classification within the Haplosclerida, prompting the adoption of molecular approaches to complement morphology in resolving haplosclerid taxonomy (McCormack et al. 2002). Thus far, studies using single nuclear gene markers, such as 18S and (primarily partial) 28S rDNA, have revealed significant discrepancies with existing morphology-based classifications. These molecular phylogenies indicate that within the five robustly identified clades (provisionally assigned 'Clades A–E'), all (morphologically defined) families and their genera are para- or polyphyletic, contradicting traditional (i.e., morphology-based) taxonomic groupings (McCormack et al. 2002; Redmond et al. 2011, 2013). Despite the consistency among molecular markers and the persistent conflict between the morphological and molecular hypotheses, no significant revisions have been attempted thus far, leaving the classification of the families and their genera unchanged and the evolution of their morphological traits unresolved.

Previous studies called for including more gene markers and using integrative morphological-molecular approaches, with emphasis on including type species, to resolve these discrepancies based on a more comprehensive phylogenetic framework (McCormack et al. 2002; Redmond et al. 2011; Morrow and Cárdenas 2015). Primary type specimens (holotypes) serve as definitive references for species identification in modern taxonomy (Erpenbeck, Ekins, et al. 2016; Derkarabetian et al. 2019; Sluys 2021). Direct comparison with holotypes offers a more objective and accurate species concept than relying on expert opinions or historical descriptions, reinforcing the original species name as proposed. However, using taxonomic reference data in phylogenomic studies has still not been fully established, especially for diverse groups at higher taxonomic levels.

In this study, we adopt a top-down approach to resolve the Haplosclerida conundrum, utilising target capture enrichment on vouchers from historical museum collections, including type specimens from 22 of the 28 genera covering the five major haplosclerid families, alongside additional contemporary specimens. van der Sprong et al. (2024) demonstrated the efficacy of target capture enrichment of UCEs in constructing a well-supported phylogeny of Red Sea Haplosclerida, consistent with previous molecular-based hypotheses (McCormack et al. 2002; Redmond et al. 2011, 2013; Erpenbeck et al. 2023). This probe assay successfully captured loci within exon (i.e., protein coding) regions across a broad sampling of Haplosclerida (van der Sprong et al. 2024). Unlike UCEs, which are highly conserved and under purifying selection, exons have an increased chance of being heterogeneous in their base composition as they are prone to synonymous or silent substitutions (Karin et al. 2020; Alda et al. 2021). Thus, while UCEs are similar across genomes and retain evolutionary signals at deeper phylogenetic nodes, exon loci can contain low levels of variation, making them better suited for capturing clade-specific signals. Therefore, to describe these loci, we introduce the term Clade-Specific Elements (CSEs) to indicate captured exon loci of a particular taxon. By taking this approach, we demonstrate that CSEs are well capable of constructing a robust phylogenomic baseline for guiding haplosclerid systematics. This opens up new opportunities for revisiting long-standing taxonomic questions and re-evaluating the evolutionary significance of morphological character states in this highly diverse and functionally relevant marine invertebrate group.

2 | Material and Methods

2.1 | Sample Collection

A comprehensive collection of sponges from the demosponge order Haplosclerida was sampled, focusing on type specimens from a wide array of genera and lower taxa across various museum collections. Taxonomic reference data was obtained from the Natural History Museum (NHM) in London, United Kingdom; the Muséum National d'Histoire Naturelle (MNHN) in Paris, France; Naturalis Biodiversity Center (RMNH/ZMA) in Leiden, the Netherlands; Staatliche Naturwissenschaftliche Sammlungen Bayerns (SNSB) in Munich, Germany; the Naturmuseum Senckenberg in Frankfurt (SMF), Germany; and the Smithsonian National Museum of Natural History (USNM), NW Washington, United States. Our sampling comprised 428 specimens, covering all 6 families and 22 genera within Haplosclerida, including 297 type specimens, among which are 115 holotypes. We searched for the haplosclerid genera and subgenera types, which we refer to as 'type species' (Table S1), to which the scientific name of an organismal group is formally attached. Of these, we sampled 13 type species of haplosclerid (sub)genera. Note that some of these type species are registered as holotype, but others as lectotype or 'type'. The sampling also included historical samples from renowned expeditions such as the HMS Challenger (1872–1876), HMS Alert (1856), and Siboga (1899–1900). The oldest specimen, *Callyspongia (Cladochalina) plicifera* (MNHN.DT.59, type) from the M. Turgot collection, was collected in 1763 in the Caribbean and identified by Jean-Baptiste de Lamarck (de Lamarck 1814). Sampling was supplemented with contemporary specimens from similar type localities as the voucher material (i.e., topotypes), with the most recent specimens collected in 2022. For subsampling, minor cuts of sponge tissue from each specimen were preserved in 98% ethanol until further processing. We documented images of the specimens and labels (all sample images and metadata are available in the Sponge Barcoding Database URL: https://sbd.palmuc.org/) and cross-referenced our findings with the World Porifera Database (https://www.marinespecies.org/porifera/).

2.2 | DNA Extraction, Library Preparation and Target Capture Enrichment

DNA was extracted from a small piece of sponge tissue using the NucleoSpin Tissue Kit (Macherey-Nagel GmbH & KG, Germany), following the manufacturer's protocol for genomic DNA from tissue samples. For samples with degraded and ancient DNA, a CTAB-based protocol for complex invertebrates was applied (Vargas et al. 2021) or the DNA extraction protocol described in Agne et al. (2022). Before library preparations, sample concentrations were assessed using a Qubit 2.0 fluorometer. High-weight DNA from well-preserved and more recently collected specimens was fragmented using NEBNext dsDNA Fragmentase Reaction Buffer v2 (New England BioLabs Inc.). Briefly, 1 μL 200 mM $MgCl_2$, 2 μL Fragmentase reaction buffer, and dH_2O were added to 1–16 μL of the sample (5 ng–3 μg of DNA) to a final volume of 18 μL and incubated for 30 min at 37°C. The reaction was stopped by adding 5 μL 0.5 M EDTA. The fragmented DNA was cleaned with SPRI beads, diluted with dH_2O to a 1:1 ratio, and eluted to a final volume of 10 μL.

Double-stranded DNA (dsDNA) libraries were prepared for each sample using a modified protocol of the xGenTM ssDNA & Low-Input DNA Prep (96 rxn) library kit (see van der Sprong et al. 2024). Libraries were quantified and quality-controlled with a Qubit 2.0 fluorometer and a Bioanalyzer 2100 in High Sensitivity DNA mode, following the manufacturer's protocol. Genomic DNA libraries were amplified to obtain 250 ng for hybridisation. A custom-made multilocus probe assay was employed to capture CSEs in Haplosclerida (van der Sprong et al. 2024). Pools of eight libraries were created based on the size of the libraries (ranging between 280 bp and 560 bp). The hybridisation and target capture enrichment were performed following the MyBaits target enrichment standard protocol. In the pilot run, the target-enriched libraries were sequenced with 150PE on an Illumina MiniSeq in high-throughput mode at the facilities of the Chair of Geobiology and Paleobiology, Department of Earth & Environmental Sciences, Ludwig-Maximilians-Universität München, Germany. Up to 32 libraries were sequenced per high-throughput MiniSeq run (i.e., four target capture pools). Another batch of libraries was sequenced with 150PE on a NovaSeq platform at Novogene (UK) Company Limited, Cambridge, United Kingdom, with a 4G raw data output per pool. For a complete overview of all samples for which DNA extractions and libraries were prepared and target capture data was retrieved, consult Tables S2 and S3, respectively.

2.3 | Data Acquisition and Assembly

The demultiplexed Illumina raw reads were quality-controlled for adapter contamination and low-quality bases by Novogene (UK) Company Limited or using *fastp* (Chen et al. 2018). This was followed by processing the clean reads using PHYLUCE: Tutorial I: UCE Phylogenomics (https://phyluce.readthedocs.io/en/latest/tutorials/tutorial-1.html) (Faircloth et al. 2012; Faircloth 2016), following the same workflow as van der Sprong et al. (2024). Briefly, data from the specimens were assembled with the command line phyluce_assembly_assemblo_spades using SPAdes version 3.15.4 (Bankevich et al. 2012).

In addition to our newly generated data, we included previously published transcriptomic datasets for 11 haplosclerid species, including *Amphimedon queenslandica*, *Haliclona (Gellius) amboinensis*, *Haliclona (Reniera) cinerea*, *Haliclona (Reniera) tubifera*, *Haliclona (Rhizoniera) indistincta*, *Haliclona (Rhizoniera) viscosa*, *Haliclona (Haliclona) oculata*, *Haliclona (Haliclona) simulans*, *Petrosia (Petrosia) ficiformis*, *Neopetrosia compacta*, and *Xestospongia testudinaria*. We also included transcriptomic data from six additional demosponges: *Latrunculia (Aciculaatrunculia) apicalis*, *Scopalina* sp., and *Tethya wilhelma* (from the same subclass as Haploscleridа, i.e., Heteroscleromorpha), as well as *Dendrilla antarctica*, *Lendenfeldia chondrodes*, and *Spongia (Spongia) officinalis* (from the subclass Keratosa). Furthermore, we added target captured sequence data from 26 haplosclerid specimens from the Red Sea (van der Sprong et al. 2024). All additional transcriptomic and target captured sequence data were previously published and sourced from publicly available repositories (see Table S4 for references and accession numbers).

2.4 | CSE Selection and Phylogenetic Analysis

To identify orthologous loci, we used phyluce_assembly_match_contigs_to_probes to match assembled contigs (derived from both newly generated and previously published sequence data) against a previously developed multilocus probe assay targeting exon regions in haplosclerids (van der Sprong et al. 2024). Only contigs matching probe sequences with a minimum of 85% identity and coverage were retained as putative CSEs for downstream analyses. The resulting loci were extracted using the command lines phyluce_assembly_get_match_counts and phyluce_assembly_get_fastas_from_match_counts. GBlocks internal trimming was performed on the CSE loci alignments using phyluce_align_seqcap_align and phyluce_align_get_gblocks_trimmed_alignments_from_untrimmed with default parameters and subsequently cleaned using phyluce_align_remove_locus_name_from_files. From these processed alignments, we first constructed five concatenated CSE datasets based on increasing thresholds of locus occupancy, ranging from 15% to 35% of taxa ($n = 228$ specimens in total) using phyluce_align_get_only_loci_with_min_taxa. Among the 228 specimens in the alignment matrices, three specimens were processed in duplicate by extracting and enriching two tissue samples from the same voucher. This technical duplication was intended to verify consistency in target capture performance and subsequent phylogenetic placement. Maximum Likelihood (ML) inference was carried out on each of the concatenated alignment matrices using RAxML version 8.2.12 (Stamatakis 2014), with the options set to rapid bootstrapping (model GTRGAMMAX, 1000 bootstrap replicates). A rogue taxon identification algorithm was applied to the resulting ML phylogenies and their associated bootstrap data using RogueNaRok version 1.0 (Aberer et al. 2013). Rogue specimens are species or sequences that exhibit unstable positions across different phylogenetic trees or bootstrap replicates, leading to reduced support values and overall instability in the inferred phylogeny (Aberer et al. 2013). Based on these assessments, we pruned taxa identified as 'rogue' to avoid ambiguous placement of the specimens in the phylogenies. In total, 29 specimens were identified as rogue based on their unstable placement across the ML phylogenies and were pruned accordingly. Of these, 17 also exhibited low CSE recovery (≤ 50 loci), which may have contributed to their phylogenetic placement instability. We then constructed five concatenated alignment matrices with a locus occupancy ranging from 15% to 35% using the reduced, filtered dataset including $n = 199$ taxa (see Table 1), and conducted new ML analyses on these optimised alignments under the same model settings as described above. In addition, we calculated the number of phylogenetic informative (PI) sites for all data matrices using PAUP* 4.0a169 (Swofford and Sullivan 2009). The analysis was followed by generating a 50% majority rule (MJR) consensus phylogram from the five ML phylogenies using Consensus Tree Builder in Geneious Prime version 2019.2.3, which served as a 'backbone phylogeny' to identify the clades that were consistently present. All phylogenies were rooted with Keratosa.

To increase the resolution within specific phylogenetic lineages, we repeated the orthologous locus identification and phylogenetic inference steps described above for each of the major clades identified in the backbone phylogeny (Figure S1). For each clade, we selected the corresponding subset of specimens and generated three new concatenated alignment datasets, based on different thresholds of locus occupancy with a minimum threshold ≥ 250 shared loci (Table 1). Because sequence similarity is generally higher among more closely related taxa, this clade-specific approach recovered more loci per dataset. For the resulting datasets, we calculated the PI sites and conducted ML (GTRGAMMAX, 1000 bootstrap replicates) and Bayesian inferences (200,000 generations, 25% burn-in) using the same phylogenetic model settings and RevBayes for the Bayesian inference

TABLE 1 | Summary statistics for concatenated Clade-Specific Elements (CSEs) alignment matrices used for backbone phylogenetic inferences and the different clades.

CSE dataset	Locus occupancy threshold (%)	#Loci	Total alignment length (bp)	#Constant sites (bp)	#PI sites (bp)	PI sites (%)	Ratio PI—variable sites (%)
Backbone phylogeny $n=228$	15	1138	181,371	64,452	94,800	53	81
	20	743	117,842	41,468	61,598	52	81
	25	450	71,896	24,981	37,615	53	80
	30	274	44,306	15,209	23,142	52	80
	35	140	23,341	7977	12,144	52	79
Backbone phylogeny $n=199$	15	1269	207,844	76,323	107,647	52	82
	20	840	135,585	48,614	70,996	52	82
	25	530	85,514	30,341	44,836	52	81
	30	350	57,098	19,866	30,027	53	81
	35	201	32,919	11,517	17,159	52	80
Clade AFG $n=75$	25	544	97,902	44,560	42,769	44	80
	30	373	66,540	30,442	29,078	44	81
	35	238	42,502	19,145	18,844	44	81
Clade BD $n=53$	25	657	144,810	72,201	50,850	35	70
	30	481	101,839	49,987	37,864	37	73
	35	307	63,572	30,875	24,633	39	75
Clade E $n=11$	35	1071	589,809	364,962	74,920	13	33
	45	844	464,642	271,240	74,920	16	39
	50	627	321,159	177,895	68,159	21	48
Clade C $n=54$	25	1147	263,684	132,240	97,339	37	74
	30	815	178,951	86,575	70,629	39	76
	35	634	136,173	65,903	54,594	40	78

Note: Each matrix was constructed based on a minimum taxon occupancy threshold. Shown are the number of CSE loci retained per matrix, total alignment length (bp), and phylogenetic-informative (PI) site information.

(Höhna et al. 2016). The resulting Bayesian trees were checked for convergence using the R package *Convenience* (Fabreti and Höhna 2022).

2.5 | Hypothesis Testing of the Genera and Families

We tested the monophyly of all genera for which we had at least two specimens with captured CSE loci, regardless of whether they appeared monophyletic or non-monophyletic in our backbone phylogeny. Genera with only a single specimen were excluded from the analysis, as monophyly cannot be assessed with a single representative. Hypothesis testing was conducted using a constrained tree analysis in IQ-TREE 2 version 2.1.4-β (Minh et al. 2020), applying the weighted Kishino-Hasegawa (KH) test (Kishino and Hasegawa 1989), the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa 1999), and the approximately unbiased (UA) test (Shimodaira 2002). Model settings were consistent with the RAxML analysis (GTRGAMMAX model), and replicates were set to 10,000. For family-level monophyly tests, we constrained the phylogenies to enforce the monophyly of families, as defined by the currently accepted morphology-based taxonomy. This enabled us to directly compare the fit of the data to our unconstrained molecular CSE-based phylogenomic hypothesis with that of the morphologically defined constraint trees at the family level.

3 | Results

3.1 | Retrieved Clade-Specific Elements (CSEs)

From the total number of samples ($n=428$) in our collection, we successfully extracted DNA from 283 specimens. These 283 specimens included 178 type specimens, of which 73 were holotypes. Among these, we identified 13 specimens as type species of their respective genera or subgenera (i.e., the reference points

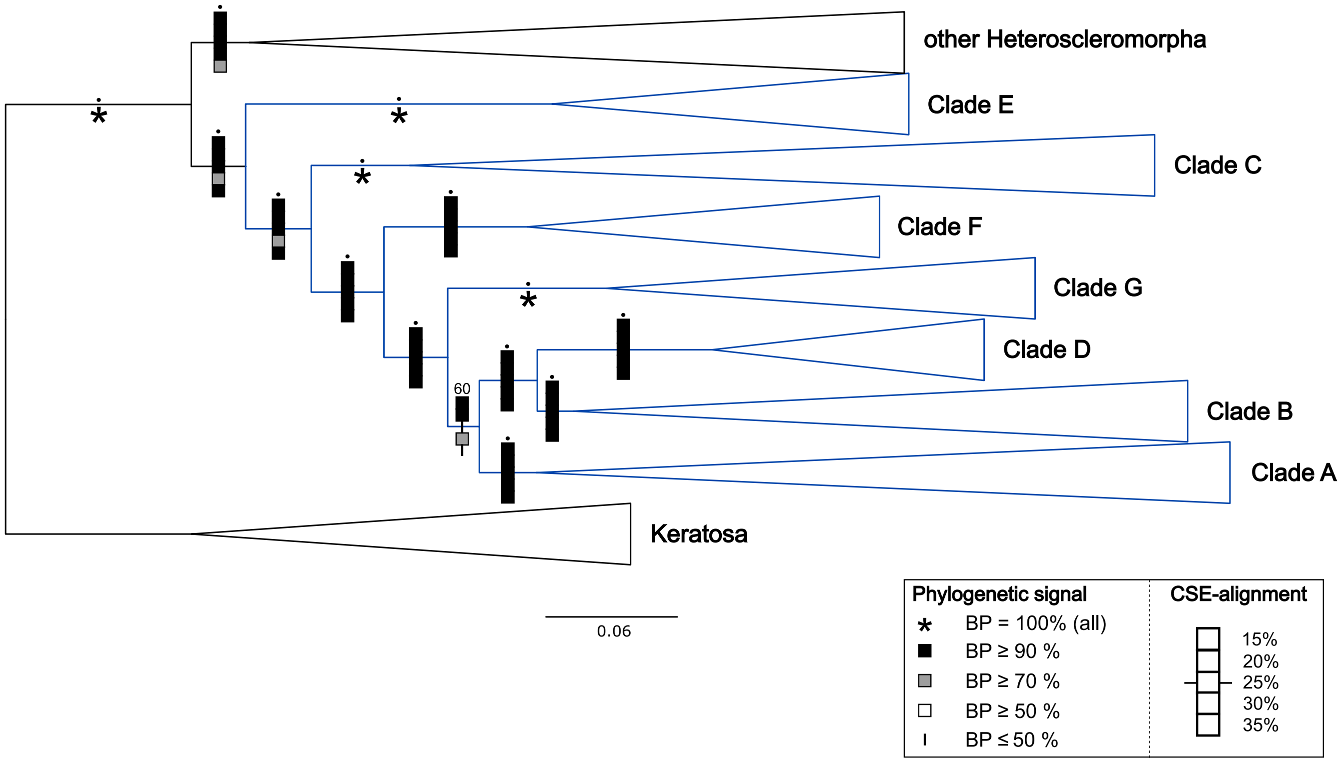

FIGURE 1 | A 50% majority rule (MJR) phylogram for the order Haplosclerida (depicted in blue), derived from the five backbone maximum likelihood (ML) phylogenies based on alignment matrices consisting including 1269 CSEs (in at least 15% of all taxa; 207,844 bp), 840 CSEs (20%; 135,585 bp), 530 CSEs (25%; 85,514 bp), 350 CSEs (30%; 57,098 bp), and 201 CSEs (35%; 32,919 bp) ($n = 199$). Consensus values (%) and bootstrap (BP) values (%) are indicated at the nodes. Nodes with unanimous BP values of 100% are marked with an asterisk (*). Consensus values of 100% are shown with a bullet (•). The clades are collapsed for clarity (see Figure S1 for the detailed phylogram). The scale bar represents substitutions per site.

for taxonomic identification). From the 283 DNA-extracted specimens, we successfully obtained 203 libraries, which comprised 114 type specimens, of which 44 were holotypes. Among these, we identified 11 type species. Finally, CSEs were successfully retrieved from 185 specimens, including 105 type specimens, of which 42 were holotypes, and we identified 8 type species among these.

The raw reads (paired) obtained from the target-captured libraries ranged from 120,216 to 13,117,402 (mean: 3,187,446 ± 2,640,136 SD). After quality filtering and adapter trimming, the number of reads ranged from 109,862 to 12,619,114 (mean: 3,123,503 ± 2,603,733 SD). The number of contigs obtained for the samples ranged from 5367 to 606,893 (mean: 77,056 ± 77,056 SD), with an average length of 270 ± 58 bp (mean ± SD, range: 48 to 70,328 bp). Using PHYLUCE, we retrieved between 7 and 1394 CSEs per sample (mean: 382 ± 249 SD). The average length of the CSE contigs varied between 602 ± 256 bp (mean ± SD, range: 56 to 18,629 bp).

After pruning 29 taxa identified as rogue, based on their unstable placement in the ML phylogenies (see also Materials and Methods), the final dataset included 199 specimens for downstream phylogenomic analysis. It consists of newly generated (156 specimens) and previously published transcriptome data from 17 demosponges and target-captured assemblies of 26 haplosclerid specimens from the Red Sea (van der Sprong et al. 2024) (see Tables S4 and S5). Among these 199 specimens were 86 types, including 36 holotypes. In the analysis, we included genomic data from 5 type species of (sub)genera: *Petrosia (Strongylophora) durissima* (NHMUK.1907.2.1.37), *Haliclona (Flagellia) indonesiae* (RMNH Por. 2326), *Dasychalina fragilis* (NHMUK.1887.5.2.170), *Pachychalina rustica* (MNHN.DT.747), and *Microxina charcoti* (MNHN.DT.691).

3.2 | Backbone Phylogeny and Clade Identification

The final dataset consisted of 199 specimens. Out of these, we constructed five alignments with different sequence coverage ranging from 15% ($n = 1269$ CSEs; 207,844 bp) to 35% ($n = 201$ CSEs; 21,919 bp). For alignment, we calculated the number and proportion of PI sites, which accounted for 52%–53% of the total sites. The ratio of PI-to-variable sites remained consistently high (79%–82%) (Table 1). The resulting 50% MJR phylogram of the five ML inferences, referred to as our backbone phylogeny, identified seven distinct ingroup clades (Figure 1 and Figure S1). Among these, we recovered the five previously established haplosclerid clades 'A–E' (Redmond et al. 2011, 2013) and two additional groups, denoted as 'Clade F and G'. Few specimens initially identified as haplosclerids were found to group outside Clades A–G alongside other heteroscleromorph sponges. These included *Haliclona pedunculata* (NHMUK.1887.5.2.233, type), *Haliclona (Rhizoniera) strongylata* (MNHN.IP.2015.292, syntype), *Oceanapia microtoxa* (ZMA Por. 11,253, holotype), *Microxina simplex* (MNHN.DT.731, 'type'), and *Petrosia* sp. (RMNH Por. 12520) (further details of the 50% MJR phylogram are provided in

Figure S1). The five ML inferences were congruent for all clades, with a consensus of 100% for almost all the nodes, except for the node where Clades A, B and D coincide, and for which we found a consensus of 60%. The nodes of the ML analyses based on the higher-coverage alignments (1269 and 840 CSEs) were supported by bootstrap values (BP) of ≥97%. Nevertheless, for alignments based on fewer CSEs, support values remained high for the majority of the nodes (≥94%), with exception of the branching point of Clades A, B, and D (BP = 83% in the phylogeny based on 350 CSEs), the branching points that separate the haplosclerid clades from the other Heteroscleromorpha and Clade E from the other haplosclerid clades (BP = 89% in the phylogeny based on 350 CSEs), and the clade containing other Heteroscleromorpha (BP = 71% in the phylogeny based on 201 CSEs). Despite these exceptions, the overall support for the backbone phylogeny remained consistent. Below, we focus on five haplosclerid clades to illustrate how CSE loci can settle longstanding classification challenges. The insights gained from these clades are representative and applicable to clades C and E (see Figures S2 and S3). Bayesian inferences computed for these haplosclerid clades all reached convergence, and the resulting topologies were consistent with the ML phylogeny for the respective clade (https://github.com/PalMuc/HaplosclerIdMuseomics).

3.3 | CSE-Based Insights Into Interrelationships Haplosclerida

Based on the backbone phylogeny, four major clades were selected for separate phylogenetic analyses: Clade AFG ($n = 75$), Clade BD ($n = 53$), Clade C ($n = 54$), and Clade E ($n = 11$). For each clade, three concatenated CSE alignments were generated using varying locus occupancy thresholds. For Clade AFG, thresholds of 25%, 30%, and 35% produced alignments containing 238–544 CSEs, with total alignment lengths of 42,502–97,902 bp, respectively. Clade BD yielded 307–657 CSEs (63,572–144,810 bp), and for Clade C this resulted in 634–1147 CSEs (136,173–263,684 bp). Due to the smaller number of taxa in Clade E, higher thresholds of 35%, 45%, and 50% were applied, resulting in alignments containing 627–1071 CSEs and spanning 321,159–589,809 bp. The proportion of PI sites ranged from 13% (Clade E, 35% threshold) to 44% (Clade AFG, all thresholds). PI site proportions for Clade BD and Clade C ranged from 35% to 39% and 37% to 40%, respectively. The ratio of PI to variable sites varied between 70% (Clade BD, 25% threshold) and 81% (Clade AFG, 30% threshold). Full alignment statistics are summarised in Table 1.

For most internal nodes in Clades A, F and G, we observed high congruence across the three Bayesian phylogenies derived from the different CSE alignments (Figure 2). The majority of nodes achieved 100% consensus with high posterior probability (PP) values of 1. In a few cases, lower consensus (66.7%) was observed at the tips of the MJR phylogram and some deeper nodes within Clade A, particularly in subgroups dominated by *Haliclona* and *Callyspongia* the Bayesian phylogeny derived from the largest CSE dataset (544 CSEs) showed overall high support values, with most nodes having PP ≥ 0.95.

In Clade A, we observed sister relationships of the holotype and paratype of *Callyspongia (Callyspongia) roosvelti* (ZMA Por. 13984 and ZMA Por. 13985, respectively), as well as for the holotype and paratype of *Haliclona (Soestella) xena* (NHMUK.1986.7.31.1 and ZMA Por. 6033, respectively). The non-type specimens *Petrosia (Petrosia) ficiformis* (RMNH Por. 12513 and transcriptome), *Petrosia (Petrosia) spheroida* (SMF.12221 and RMNH Por. 9541), and *Haliclona (Reniera)* aff. *fascigera* (RMNH Por. 11668 and RMNH Por. 11661) grouped with specimens with the same scientific name. An exception was found for *Callyspongia (Callyspongia) siphonella* (ZMA Por. 17338), whose type specimen was separated from its congeners (SNSB-BSPG.GW3010 and SNSB-BSPG.GW3123). Some notable placements were those of *Cladocroce guyanensis* (RMNH Por. 9826, holotype, Family Chalinidae), *Niphates lutea* (ZMA Por. 13578, holotype, Family Niphatidae), and *Calyx magnoculata* (RMNH Por 9254, holotype, Family Phloeodictyidae), which ended up in the two subclades mainly dominated by the Petrosiidae genera *Petrosia* and *Neopetrosia*. Also, a species belonging to the demosponge order Axinellida, *Parahigginsia strongylifera* (RMNH Por. 9251, holotype), was observed among specimens of the genus *Petrosia*.

Based on the different numbers of loci included in the phylogenetic analysis, a few discrepancies were observed between the Bayesian inferences. The holotype of *Haliclona (Soestella) xena* (ZMA Por. 5000) grouped differently from its paratypes (ZMA Por. 6033 and NHMUK.1986.7.31.1), aligning with *Haliclona (Reniera) cinerea* in the phylogeny based on 544 loci. Similarly, the specimens of *Callyspongia (Callyspongia) siphonella* (SNSB-BSPG.GW3010 and SNSB-BSPG.GW3123) showed shifting placements, either clustering with species of *Haliclona* and *Chalinula* in the phylogeny based on 544 CSEs or forming a sister group with *Callyspongia* and *Haliclona* based on the phylogenies, including 373 and 238 CSEs.

For Clades B and D, we observed a consensus of 100% for most of the nodes (Figure 3). Lower consensus values occurred due to the varying placement of a few specimens, dependent on the number of CSEs used in the Bayesian inference. This concerned *Microxina charcoti* (MNHN.DT.691), *Amphimedon* aff. *texotli* (RMNH Por. 10931), and the internal relationships between specimens identified as *Neopetrosia carbonaria* (RMNH Por. 12,156, RMNH Por. 12491, and RMNH Por. 12343). The Bayesian inference based on the most loci (657 CSEs) showed high support values (PP = 1), except for one node within Clade B. The support values for some of the nodes in the Bayesian inferences based on fewer loci (i.e., 481 and 307 CSEs) were moderate to low in a few cases. Clade D exclusively contains specimens of the genus *Xestospongia*. We observed consistent clustering of *Xestospongia arenosa* (ZMA Por. 6404, holotype; ZMA Por. 6409, paratype) and *X. muta* (RMNH Por. 12516; RMNH Por. 12515). Specimens identified as *X. testudinaria* (RMNH Por. 12503; RMNH Por. 12522 RMNH Por. 12519; SNSB-BSPG.GW3122; SNSB-BSPG.GW4191; SNSB-BSPG.GW4374) were found to be non-monophyletic, as this subgroup also included *X. variabilis* (NHMUK.1887.5.2.214) and *X. portoricensis* (ZMA Por. 3728, paratype). In Clade B, taxon-specific patterns were less clear, particularly at higher taxonomic levels, as various members from the five major families were non-monophyletic. However, at the species level, we observed sister relationships for most specimens, such as the holo- and paratype of *Haliclona (Halichoclona) vansoesti* (ZMA Por. 13391 and ZMA Por. 13394),

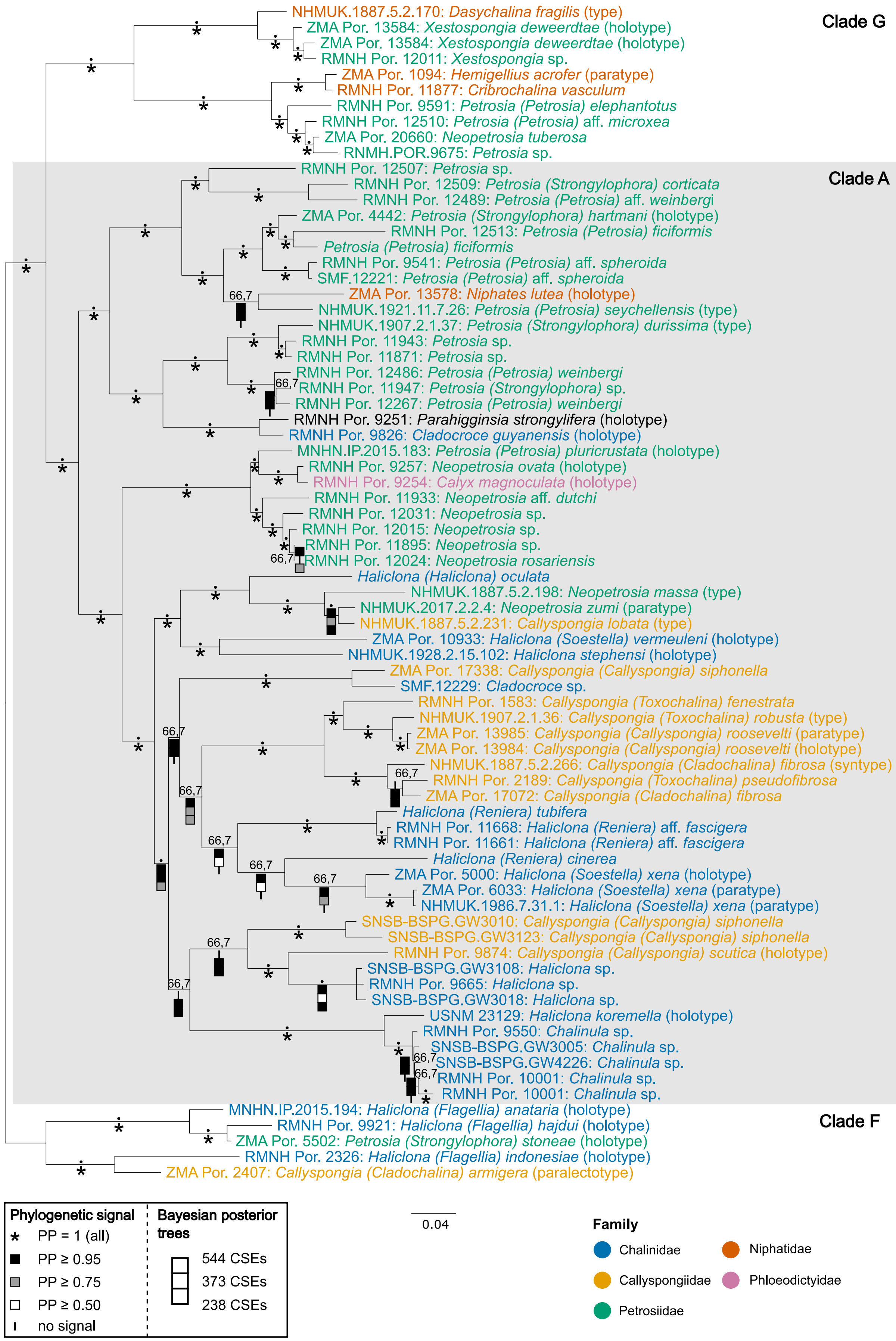

FIGURE 2 | Legend on next page.

FIGURE 2 | A 50% majority rule (MJR) phylogram for Clade A, F and G, configured from three Bayesian phylogenies based on alignment matrices including 544 CSEs (97,902 bp), 373 CSEs (66,540 bp), and 238 CSEs (42,502 bp). The phylogram is rooted to Clade F ($n = 75$). Consensus values (%) and posterior probability (PP) values are provided at the nodes, with nodes displaying unanimous PP = 1 marked by an asterisk (*). Consensus values of 100% are indicated with a bullet (•). The museum number precedes taxon names; specimens lacking a museum number were sourced from genome projects (see Table S4). The species indicated in black is currently not classified as haplosclerid. The scale bar represents 0.04 substitutions per site.

the syn- and lectotype of *Hemigellius acrofer* (ZMA Por. 1093 and ZMA Por. 6075), as well as for non-type material, see for example *Arenosclera arabica* (SNSB-BSPG.GW4246, SNSB-BSPG.GW4146, and SMF.12222). The only exception was found for the holo- and paratype of *Haliclona (Halichoclona) stoneae* (ZMA Por. 15886 and ZMA Por. 13502, respectively).

3.4 | Hypothesis Testing

The hypothesis test, performed to compare the monophyly of taxa based on morphology-based classification against our CSE-based hypothesis, showed that only the monophyly of *Acanthostrongylophora* (p-AU = 0.288) and *Dendroxea* (p-AU = 0.577) could not be rejected according to the AU test. This finding aligns with our tree topology, where these two genera were also found to be monophyletic. For the remaining 16 genera—*Amphimedon, Arenosclera, Callyspongia, Chalinula, Cladocroce, Cribrochalina, Dasychalina, Haliclona, Hemigellius, Microxina, Neopetrosia, Niphates, Oceania, Petrosia, Siphonodictyon*, and *Xestospongia*—their monophyly was rejected (p-AU values < 0.05; see Table S6A for detailed results). The topologies for which monophyly was tested at the family level were all rejected by the AU test (Table S6B).

4 | Discussion

Effective communication about species identity and biodiversity relies on unambiguous taxonomic identification (Wheeler 2004). Taxonomic names reflect the structure of the phylogenetic framework and convey information about ancestry, diversification, and adaptation. This, in turn, relies on identifying shared derived characters (synapomorphies) among members of a monophyletic group, where individuals within a given taxonomic rank are more similar to each other than those outside that group (Hennig 1965). Our phylogenetic analysis of Haplosclerida, focusing on a comprehensive sampling of type material from natural history collections (to our knowledge, the largest approach of this kind in marine invertebrates) and contemporary specimens, provides evidence and high support that the current nomenclature and classification schemes are inadequate. Previous studies based on single gene markers have already revealed these inconsistencies. However, now based on hundreds of loci, we provide new insights into taxonomic assignments and an improved framework for re-evaluation of the current classification. The classification for Haplosclerida in its present form leads to ambiguous taxonomic boundaries that provide little to no insight into what a taxon within this group truly represents. As a result, effective communication about the identity of a taxonomic group, its members, and their ecological roles is hindered. This has consequences for interpretations of biological and ecological observations, as these are fundamentally based on what an evolutionary unit constitutes (Mayden 2002).

We demonstrate that target enrichment of CSEs is an effective method for establishing a robust genomic reference framework and re-evaluating current classification within this complex group of marine invertebrates. Our approach, which resolved clades and verified specimen placement, reveals a strong consensus and highly supported phylogenetic signals across most nodes of the Haplosclerida backbone phylogeny (Figure 1, Figure S1), as well as within the phylogenies of the individual clades (Figures 2 and 3; see also Figures S2 and S3). Most specimens have stable placements across the different phylogenies, demonstrating consistency regardless of the amount of genomic data included in the analyses (see also Table 1). Variations in placement were observed only in a few cases. This was dependent on the CSE subset used, which, subsequently, influenced the consensus and support of specific branches.

The high level of agreement in the backbone phylogeny and the individual haplosclerid clades validates the placement of the specimens. Seven haplosclerid clades were identified, including five previously recognised clades (A–E) (Redmond et al. 2011, 2013) and two novel clades, here designated as 'Clade F and G'. The specimens present in Clade F were not present in the phylogeny of Redmond et al. (2013). In Clade G, however, the species *Dasychalina fragilis* was also present in the haplosclerid phylogeny of Redmond et al. (2013). However, it likely concerns a different specimen (see GenBank accession ID DQ927316). The difference in placement of *D. fragilis* in Clade D (Redmond et al. 2013) and Clade G (this study), can be well explained by the proximity of these two clades in our backbone phylogeny. In addition, in Redmond et al. (2013), the taxon sampling of the Haplosclerida is limited compared to this study. Our data resolves Clade G as a distinct and well-supported lineage, suggesting that additional phylogenetic structure exists within this region of the haplosclerid tree, which has not been fully covered before.

Building upon earlier single-locus molecular studies, we advocate for abandoning the current morphology-based classification of Haplosclerida, and recommend that taxonomic revisions be guided by a broad genomic framework. This will offer a stable and objective baseline for understanding evolutionary relationships and species identities within this taxonomically problematic group. Due to the pervasive polyphyly of the currently morphologically recognised families, we propose to formally abandon these family names in ongoing classification efforts. Until type species can be included (or redefined where necessary), we advocate for a provisional system using designations such as '*Incertae sedis* Clade [A–G]' (see also Redmond et al. 2013). This acknowledges current phylogenetic evidence while also maintaining taxonomic transparency and flexibility. In the same vein, naming subclades and using them as provisional genera would be a pragmatic step forward, providing an improved depiction of the evolutionary structure until formal revisions are made.

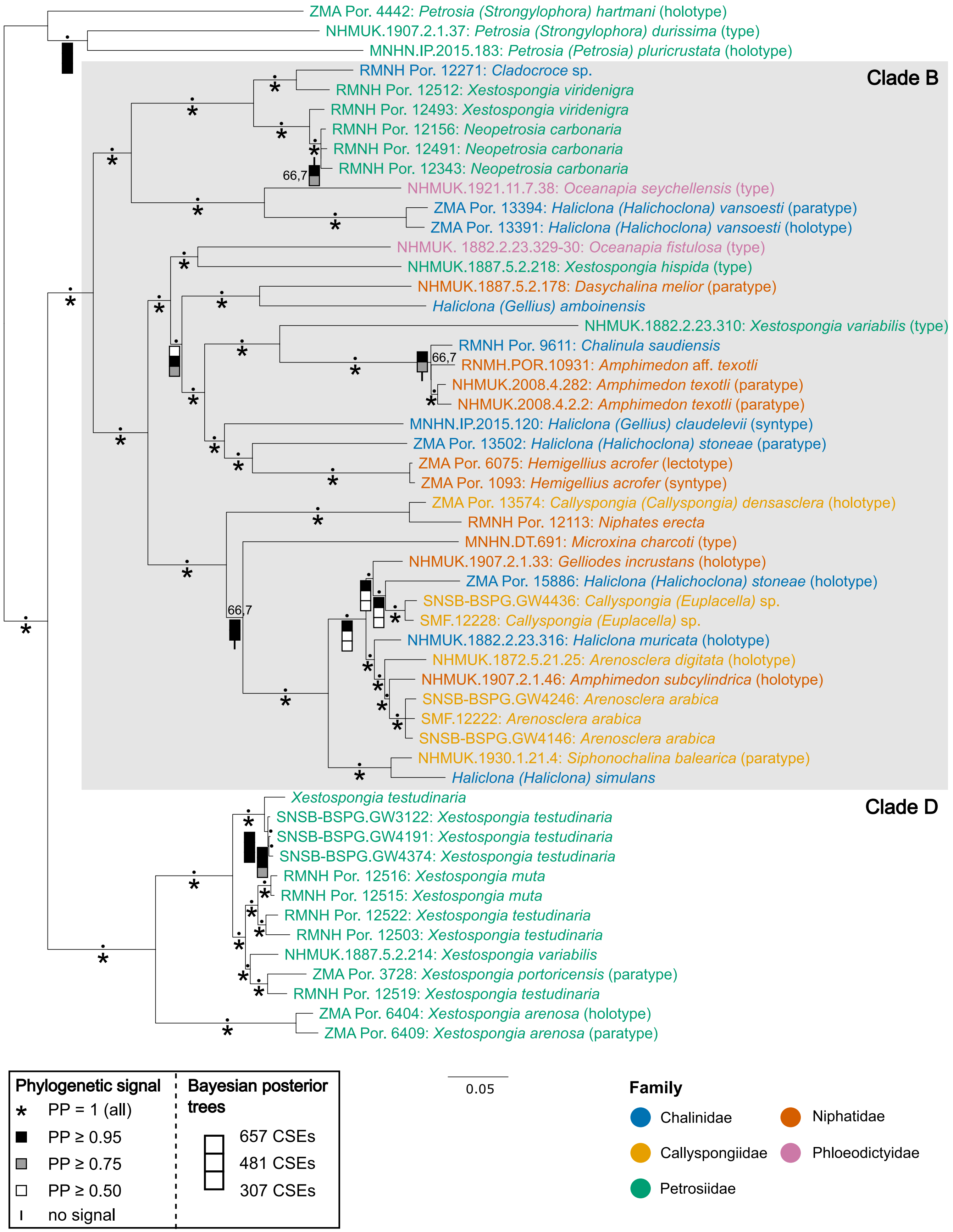

FIGURE 3 | Legend on next page.

FIGURE 3 | A 50% majority rule (MJR) consensus phylogram for Clade B and D, rooted with three specimens from Clade A ($n=53$), derived from three Bayesian phylogenies based on CSE-alignment matrices including 657 CSEs (144,810 bp), 481 CSEs (101,839 bp), and 307 CSEs (63,572 bp). The phylogram is rooted with Clade F. Consensus values (%) and posterior probability (PP) values are shown at the nodes, with nodes having unanimous PP = 1 marked by an asterisk (*). Consensus values of 100% are indicated with a bullet (•). The museum number precedes taxon names; specimens lacking a museum number were sourced from genome projects (see Table S4). The scale bar is 0.05 substitutions per site.

4.1 | CSE-Informed Limitations of Morphology-Based Classification in Haplosclerida

One of the main difficulties in sponge taxonomy is assigning species to taxonomic categories based on the very few available morphological characteristics. Because many individuals often possess traits that deviate from what is considered 'diagnostic' for a given taxon, insecurity in their classification is standard practice. Morphology-based classification systems often fail to capture the full extent of evolutionary relationships between species in non-model organisms such as Haplosclerida. For instance, *Cladocroce guyanensis* (Chalinidae, Clade A) was tentatively placed within the genus *Cladocroce* due to its thin-bladed growth form. This initial placement was made with some hesitation, as the specimen also displayed overlapping features with other genera, such as longitudinal tracts and unispicular reticulation typically associated with *Haliclona* (van Soest 2017). Similarly, *Niphates lutea* (Niphatidae, Clade A) exhibits morphological traits closely resembling those of *Petrosia* (*Strongylophora*) (Petrosiidae), including characteristics such as (i) strongyles (spicules with rounded tips) as the primary spicule type, (ii) a reticulate skeleton with spongin-embedded tracts, (iii) confused tangential reticulation in the ectosome, (iv) elastic yet firm consistency, and (v) irregular spicule distribution in the choanosome (Lehnert and van Soest 1999). Despite these similarities, *N. lutea* was eventually assigned to *Niphates* due to the absence of the two size categories of strongyles considered typical of *Petrosia (Strongylophora)* (Lehnert and van Soest 1999). A further example is *Petrosia (Strongylophora) stoneae* (Clade F), which was assigned to the subgenus *Strongylophora* based on its similarity in habitus to *P. (Strongylophora) hartmani*. However, the lack of kidney-shaped microstrongyles ascribed to the subgenus *Strongylophora* (van Soest and Stentoft 1988) was not considered. There are also instances where specimens assigned to the order Haplosclerida do not align with any of the seven identified clades (A–G) in our molecular phylogenies; instead, they group with other Heteroscleromorpha (Figure S1). The syntype of *Haliclona (Rhizoniera) strongylata* (Chalinidae) appears closely related to the non-haplosclerid *Monanchora* sp. (Order Poecilosclerida; Figure S1). Lévi and Lévi (1983) expressed difficulty in classifying *H.* (*Rhizoniera*) *strongylata* because of similarities with other species assigned to genera belonging to Poecilosclerida, such as *Strongylacidon*, *Batzella*, and *Inflatella*. Nevertheless, the species was provisionally placed in the haplosclerid subgenus *Rhizoniera* (Lévi and Lévi 1983). In all these cases, taxonomic decisions are shaped by the subjective weighting of specific traits, often prone to ambiguous or unsure taxonomic assignments. These subjective interpretations of morphological character evolution complicate classification and create an inconsistent taxonomic framework that is also used as a reference by others.

However, this does not mean morphological insights cannot be valuable for taxonomic classification or should be ignored; we have to face that the utility is often limited (at least for this group) as a result of inconsistent recognition and varying interpretations (see, for example, the descriptions of *Callyspongia crassa* (i.e., *Sclerochalina crassa*) in Keller 1889 and Lévi 1965). Despite centuries of taxonomic efforts on haplosclerids, identifying reliable characteristics for classification remains a notoriously difficult task. In general, sponge taxonomy requires a nuanced understanding that is challenging to convey in species descriptions, which, in addition, often also lack standardisation. This lack of consistency not only hampers accurate classification but also introduces biases that skew taxonomic decisions.

Other ways in which bias can be introduced, confusing taxonomic decision making, include the specimen's geographic origin or habitat. An example of this issue can be seen in *Calyx magnoculata* (Phloeodictyidae) in Clade A, which was initially assigned to the genus *Calyx* based on its dense armour of spicules (van Soest et al. 2014). Our analysis reveals that *C. magnoculata* is more closely related to *Neopetrosia ovata* (Petrosiidae) and should be reassigned to *Neopetrosia* within the family Petrosiidae. These two specimens share more phylogenetic and structural similarities than previously recognised. Likely, the ecological disparity of these sponges, in combination with some subtle morphological differences, may have led to the initial misclassifications. Namely, *C. magnoculata* was collected from a vertical limestone wall in Bonaire, whereas *N. ovata* inhabits sandy bottoms near Klein Curaçao (van Soest et al. 2014). Genetically informed phylogenies can help to avoid misconceptions about the origins and kinship of specimens, as they provide an unbiased representation of close relationships that would otherwise go unnoticed.

Another persistent challenge in sponge taxonomy is that species assignments rely occasionally on the presence (or absence) of a single, non-exclusive morphological trait; an issue extending beyond species-level classifications. Take, for instance, certain observed diagnostic characteristics which can appear atypical for specific groups. The classification of the non-haplosclerid *Parahigginsia strongylifera* (Clade A) into the genus *Parahigginsia* was initially based on a single characteristic: an ectosomal cover composed of thinly curved acanthomicroxeas—small, spiny spicules or microscleres (van Soest et al. 2014) which have not been associated with Haplosclerida. Our analyses unambiguously recover *P. strongylifera* within Haplosclerida, nested in Clade A. Van Soest et al. (2014) already noted uncertainties regarding the taxonomic placement of *Parahigginsia*, acknowledging that its reassignment from Heteroxyidae to Stelligeridae was based on limited molecular evidence. At the time, van Soest et al. decided to retain the genus in Heteroxyidae until more comprehensive data would become available. Now, based on new genomic insights, we can propose the transfer of *Parahigginsia* to the order Haplosclerida.

Another issue is that diagnostic characters that define a genus or family can be easily overlooked, or cannot be observed due to secondary loss (Leal et al. 2023; Vargas et al. 2013; van Soest and Stentoft 1988). Based on molecular phylogenetic evidence, *Oceanapia bartschi* (of the haplosclerid family Phloeodictyidae) was molecularly reassigned to *Coelocarteria* (Order Poecilosclerida, Figure S1) (Leal et al. 2023). One of the main defining characteristics of the genus *Coelocarteria* is the presence of chelae, which are C-shaped microscleres with anchor-like hooks at the tips. These chelae were not observed in *C. bartschi*, suggesting that this diagnostic trait is no longer preserved in the respective lineage (Leal et al. 2023). Our data corroborate this finding, as *C. bartschi* was also placed close to other poecilosclerids in the backbone phylogeny (Figure S1). The placement of *Oceanapia microtoxa* close to *C. bartschi* suggests that *O. microtoxa* also belongs to *Coelocarteria*. Perhaps a more detailed examination of *O. microtoxa* for traits like chelae may support its reclassification, but there is a fair chance that the character may no longer be present. Cases such as these illustrate how the absence of a single morphological trait can result in significant taxonomic misplacements, even when it concerns lineages belonging to entirely different orders. It also highlights how easily convergent evolution, homoplasy and character reduction can mislead taxonomic interpretation, and blurr phylogenetic relationships when informed by morphology alone (Fromont and Bergquist 1990; Jenner 2004; Dohrmann et al. 2006; Morrow et al. 2013). In theory, using multiple diagnostic characters can mitigate the effects of homoplasy, but for groups such as sponges, these are often hard to find due to phenotypic simplicity and plasticity.

While the limitations of morphological characters for taxonomic classification are evident, we acknowledge that a certain level of congruence between morphology and molecular data can still be observed. Within the identified clades A–G, subclades are often dominated by specific genera: Clade A contains subclades primarily composed of *Petrosia* and *Neopetrosia* (Petrosiidae), while *Haliclona*, *Chalinula* (both Chalinidae), and *Callyspongia* (Callyspongiidae) are represented in other subclades. For Clade D, this is even more pronounced since it consists exclusively of the genus *Xestospongia*. The congruence observed within Clade A, where three species of *Callyspongia*—two specimens of *C. (Cladochalina) fibrosa* and *C. (Toxochalina) pseudofibrosa*—nested within a single subclade, demonstrates that molecular data verifies specific morphological observations and offers more detailed insight. These specimens are unified by their fibrous tissue structure (Ridley and Dendy 1886). In this case, genomic data provides a more objective insight into what characteristics distinguish the subgenera than traditional morphological approaches have achieved thus far. Thus, when carefully analysed and interpreted at the lowest taxonomic level, morphological characters can make meaningful contributions to taxonomic efforts. This is also observed in the CSE-based phylogenies, where most morphologically similar (or even indistinguishable) specimens are found closely together. However, this resolution rapidly diminishes at supra-specific levels. Non-monophyly remains prevalent for most groups, indicating that morphological traits (or at least those that are currently recognised) lose their phylogenetic reliability beyond the species level.

4.2 | Taxonomic and Systematic Re-Evaluation Based on Type Species CSEs

Including genomic information from historical type material is arguably one of the most critical steps for constructing reliable phylogenies, as types provide the definitive reference for linking species names to taxa and anchoring evolutionary relationships in accurate taxonomy (Erpenbeck, Ekins, et al. 2016). The successful recovery of genomic data from type specimens using methods such as target-capture can overcome the limitations of single-marker methods, which often rely on intact, longer DNA sequences (McCormack et al. 2017). To enable re-evaluation of haplosclerid classification, we prioritised the inclusion of genomic data from as many type species as possible (Table S1) and aimed to include those at the genus level and below. Despite our efforts, various challenges, such as insufficient DNA quality, short library insert sizes, and co-extraction of DNA from other eukaryotes (particularly fungi), limited usable data for certain key specimens (Tables S2, S3, and S5). Although high-quality genomic data were obtained for some type species, many attempts yielded incomplete or poor-quality data. It should be noted that quality in this context is not necessarily defined by the number of sequencing reads obtained for a sample, nor by the number of recovered loci. Rather, it is the quality of the recovered loci that is most relevant, which can be observed for samples that consistently occupy stable positions in phylogenetic trees (see Table S5 for a full overview of the read output and CSE retrieval for each of the samples). For those type species for which we managed to recover data, we eventually gained new insights for re-examining several taxonomic groups and suggest possible revisions to improve current classifications.

Within one of the subclades of Clade A, the type species for the subgenus *Strongylophora*, *P.* (*Strongylophora*) *durissima* (NHMUK.1907.2.1.37), falls alongside other members of the genus *Petrosia*. This subclade also comprises additional type specimens, such as *P.* (*Strongylophora*) *hartmani* (ZMA Por. 4442, holotype) and *P.* (*Petrosia*) *seychellensis* (NHMUK.1921.11.7.26, type), as well as non-type material, including *P.* (*Petrosia*) *ficiformis* from the Mediterranean (Riesgo et al. 2012). These findings suggest that this subclade represents a coherent group to be classified under *Petrosia*. To further support this designation, genomic data from the lectotype of *P. ficiformis* (LMJG 15330), which was not included in this study, could be analysed in future research. Reclassifying this subclade under *Petrosia* would necessitate a broader revision of species currently assigned to *Petrosia* but positioned outside this subgroup of Clade A, as well as the re-evaluation of specimens within this clade and currently not assigned to this genus. Additionally, our data suggest that the distinction between the two subgenera, *Petrosia* (*Petrosia*) and *Petrosia* (*Strongylophora*), should be abandoned. We observe that specimens from both subgenera group closely together, indicating that the morphological characters traditionally used to distinguish these subgenera (i.e., skeletal architecture, spicule arrangement, surface texture, and slight differences in consistency) do not reflect their evolutionary relationships (Figure 2).

For another subgroup within one of the newly defined clades, denoted as Clade G, we found strong evidence supporting a taxonomic revision of the genus *Dasychalina*. This subclade includes the type species of *Dasychalina*, that is, *D. fragilis* (NHMUK.1887.5.2.170), along with three specimens currently assigned to the genus *Xestospongia*, including the holotype of *Xestospongia deweerdtae* (ZMA Por. 13584). Based on the presence of the *Dasychalina* type species within this subclade, we propose reclassifying these *Xestospongia* specimens under *Dasychalina*. In contrast, Clade D exclusively consists of specimens assigned to *Xestospongia*, suggesting that this clade represents the core group of this genus. With *Xestospongia* specimens presently being distributed across five of the seven identified haplosclerid clades, genomic data from the type species *Xestospongia diprosopia* (USNM 21509, not included in this study) could conclusively determine which clade represents the 'authentic' *Xestospongia* lineage and which specimens should be reassigned to other groups. Nevertheless, our current findings indicate that the specimens within Clade D likely represent the true members of *Xestospongia*.

Despite obtaining genomic data from three additional type species, proposing taxonomic revisions for some groups remains premature due to unresolved phylogenetic relationships and limited data. For example, the type specimen *Haliclona (Flagellia) indonesiae* (RMNH Por. 2326) was recovered in Clade F alongside two other species of the same subgenus. The complex and unresolved phylogenetic relationships within the umbrella genus *Haliclona*, which spans multiple clades (A, B, C, and F; Figures 2 and 3 and Figure S3), make it inappropriate to propose a definitive revision of Clade F solely based on this subgenus affiliation. Similarly, our analyses of *Pachychalina rustica* (MNHN.DT.747, type, Clade C) and *Microxina charcoti* (MNHN.DT.691, type, Clade B) led to inconclusive results due to the limited genomic loci (90 and 57 CSEs, respectively) and their ambiguous placements. While *P. rustica* in Clade C appeared closely related to *Dactylia dichotoma* (NHMUK.1886.27.61, type) in two out of three Bayesian inferences, the phylogeny based on a broader set of loci (1147 CSEs) indicated a closer relationship to a subgroup containing *Haliclona (Rhizoniera) indistincta* and the type specimens of *Haliclona (Reniera) griessingeri*. The type species *M. charcoti* in Clade B forms a distinct and isolated branch. Because of its distinct placement and the limited available loci, we cannot confidently propose taxonomic revisions for this specimen yet. There is, however, a small caveat, as a reduced number of loci does not necessarily result in poorer phylogenetic resolution. For example, although we only obtained 33 CSEs for *P. (Petrosia) seychellensis*, the position of this specimen remained stable (see Clade A, Figure 2). Hence, another possible explanation for the uncertain placement of these specimens could also be incomplete taxon sampling (Quattrini et al. 2018).

The broader issue is that challenges persist when type material is severely degraded, contaminated, or only available as microscopic slides, hindering molecular analyses and taxonomic verification. Where type specimens cannot provide enough information for reliable taxonomic revision, alternative approaches can be considered, such as the use of 'topotypes' (specimens collected from the same locality as the original type and showing consistent morphology). Topotypes provide an alternative to link names to genetic lineages when genomic data from the original type is unavailable. This method has been applied successfully in a recent study on the coral genus *Acropora* (Bridge et al. 2023). However, relying on morphological similarity is not without risks, especially in groups such as (haplosclerid) sponges, where morphological similarity does not seem to correspond well to genetic lineages. This can lead to misinterpretations if morphological traits have evolved convergently, show substantial plasticity, or are subject to secondary loss. Swierts et al. (2017) demonstrated that multiple genetically distinct, yet morphologically cryptic, lineages of *Xestospongia* can coexist in the same geographical area. Furthermore, some of these sympatrically formed lineages were more closely related to lineages in different ocean regions than to each other (Swierts et al. 2017). Thus, specimens collected from the same reef as the original type may actually belong to completely different and distant evolutionary lineages, which demonstrates the limitations of using morphology-based topotypes in such taxa. An alternative approach would be to use a 'genometype': a genomically cross-validated reference specimen. In such cases, a genetic reference specimen is selected based on available genomic loci of the original type specimen. When a degraded type species contains enough genomic loci for partial comparison, it can serve as a benchmark for identifying genetically corresponding specimens (Roycroft et al. 2022). Ideally, both topo- and genometypes should be adopted in tandem, with topotypes collected from the type locality serving as morphological proxies, while partial genomic data recovered from the original type specimen can help further validate the taxonomic identity of those proxies. Unlike the single use of topotypes, genometypes rely on direct genetic continuity rather than morphological resemblance only, which mitigates the risk of misclassification due to morphological ambiguities. However, this approach requires that the original type specimen contains at least some recoverable loci to serve as a genomic anchor to facilitate the identification of the genometype.

As Erpenbeck, Ekins, et al. (2016) argued, molecular identifications should always be performed in parallel with genetic-based evidence from holotypes to establish a reliable baseline for systematic research and to prevent over-reliance on non-type specimens that may introduce systematic biases. While our study supports the potential of modern methods, such as target capture, to recover genome-wide loci from older and fragmented type material, we also recognise that the successful recovery of partial genomic data cannot always be guaranteed. In some instances, we need to accept that type species will not give conclusive answers, and that designating new types for those groups for which no genomic-reference data can be obtained, must be reconsidered. Such an approach demands careful attention and close collaboration between molecular biologists, taxonomists, and museum curators to assure any new designations are stable and widely accepted. In addition, even when genomic data can be successfully retrieved from type species, caution is still warranted. Genomic-validated types bear the risk of being too readily interpreted as the 'holy grail' of a species' identity (both genetically and morphologically), while in reality they are a snapshot in time of a group with a potentially wide range of phenotypic variation. In groups plagued by phenotypic plasticity, this can lead to overly narrow species hypotheses and the exclusion of genetically similar individuals that deviate morphologically due to environmental or developmental factors. The consequence is that genomic-validated type specimens, while invaluable, may still narrow our understanding of species boundaries if treated uncritically as morphological standards.

To circumvent this, population-level sampling would be an option, as it allows for the assessment of intraspecific morphological and genetic variation across ecological and geographic gradients. But also probabilistic frameworks, such as coalescent-based species delimitation, can aid in objectively identifying species boundaries. In these approaches, genome-wide data can be integrated across multiple individuals per putative lineage to estimate lineage divergence, while accounting for ancestral polymorphisms and gene tree discordance (Fujita et al. 2012). When applied in conjunction with genomic data from type material, this can provide a statistically grounded and biologically informed basis for taxonomic decisions.

4.3 | There is No Other Way: We Need to Abandon Strictly Morphology-Based Classification for the Haplosclerida

Historically, traditional morphological classifications have provided a foundation for taxonomic understanding. However, due to their limitations in complex taxonomic groups such as Haplosclerida, a paradigm shift toward genomic data as the primary focus of classification is essential. Rigid adherence to morphological classification has often resulted in misclassifying species or higher taxa due to ambiguously defined taxonomic boundaries. Consequently, communication about species identity is undermined, affecting meaningful interpretations of ecological and experimental observations. Our genomic-based findings support that traditional morphological characters often fail to reflect evolutionary relationships in sponges (Wörheide and Erpenbeck 2007). Morphological characters must correspond to underlying genetic similarities to be taxonomically informative (Fromont and Bergquist 1990; Avise 2012), which can only be validated through molecular phylogenetics. At higher taxonomic levels within Haplosclerida and even beyond, symplesiomorphy and convergence of morphological characteristics emphasise the prevalence of homoplasy, as a result of convergent evolution or secondary loss. This issue is not exclusive to sponges but touches a broader limitation observed in other eukaryotic groups, where morphological simplicity or the conservation of traits limits the accuracy of phylogenetic interpretations. In groups such as placozoans and choanoflagellates, acceptance is rising that morphology is not key in taxonomy and classification, and studies have revealed previously unrecognised diversity based on genomic information that would otherwise have stayed unnoticed (Muggia et al. 2011; Carr et al. 2016; Schiwitza et al. 2018; Eitel et al. 2018; Steins et al. 2023). For example, genomic analyses have shown that placozoans, despite their simple morphology, consist of multiple deeply divergent lineages (Eitel et al. 2024). Likewise, the conserved morphology of choanoflagellates has proven inadequate for phylogenetic classification, while genetic, functional, and ecological data have resulted in more precise insights into their relationships (Carr et al. 2016; Schiwitza et al. 2018). The lessons learned from these groups accentuate the value of a genomic approach for solving taxonomic complexity in morphologically cryptic lineages such as Haplosclerida. Our study demonstrates that the morphological characteristics in haplosclerids, particularly at higher taxonomic levels, are unreliable for distinguishing taxa. This may be due to them being overlooked, gaps in our current knowledge, or because we lack the technology necessary to detect them. For complex groups such as Haplosclerida, genomic evidence offers, therefore, a more stable foundation for understanding species relationships, unlike the current morphology-based classification. By prioritising genotypic data, an objective framework can be provided that more accurately represents evolutionary relationships and guides the re-evaluation of (morphological) synapomorphies. This shift is not just necessary but also essential for ensuring that taxonomic classifications provide a meaningful basis for ecological and evolutionary studies across biological disciplines.

5 | Conclusion

In this study, we demonstrate the high potential of target capture enrichment of CSEs from taxonomic reference material to address longstanding systematic issues within the enigmatic demosponge order Haplosclerida. Using CSE-based data obtained from the largest sampling of Haplosclerida to date, including historical type material alongside contemporary specimens, we provide a robust phylogenetic framework that better reflects the evolutionary relationships than the current morphology-based classification schemes. Our findings are consistent with previous molecular hypotheses but also provide new insights into the pitfalls that arise from adhering to morphology-based classification. We expose how homoplasy, convergent evolution, and secondary loss of morphological characters often lead us astray when it comes to taxonomic decision-making. This also applies to the geographical occurrence of a specimen or their ecology, which can influence our judgement about their taxonomic identity. Consequently, we advocate for a transition from morphology-based decisions toward a genomic-informed approach. Applying genome-wide data can circumvent taxonomic biases, as it provides a more robust phylogenetic hypothesis and baseline that places kinship in an objective perspective. Furthermore, it gives us the opportunity to identify those properties that are genuinely synapomorphic. This may include previously overlooked morphological characteristics, as well as other relevant traits such as the microbial communities or ecology. To further facilitate revision of this group, it is crucial to apply genomic data derived from historical type material, as this provides the best possible taxonomic reference framework. Based on the genomic evidence, we propose the reassignment of the genera *Petrosia*, *Xestospongia*, and *Dasychalina*. However, we also see potential for further improvement. For example, the designation of 'genometypes' for those type species for which very little genomic data can be obtained, preferably in tandem with a topotype approach. If genetic material can no longer be retrieved, a new type must be designated, but we acknowledge that this would require close collaboration between molecular biologists, taxonomists, and curators. Using an approach that includes considerably more genetic information than before and largely based on historical type material, we now demonstrate that relying on the morphology-based system to identify Haplosclerida subtaxa offers little insight into their evolutionary context and role, and argue that genotypically informed phylogenies are the only way forward.

Author Contributions

J.v.d.S., S.V., D.E. and G.W. are part of the DFG-funded promotion project, and S.V., D.E. and G.W. are responsible for the project's design and research supervision. This included assisting in interpreting the data

and writing the paper. J.v.d.S. was accountable for collecting taxonomic reference data from natural history collections. J.v.d.S. contributed to the laboratory work, performed bioinformatic analysis of the acquired data, and wrote the initial manuscript draft. D.E., S.V. and G.W. provided significant contributions to the manuscript. S.S. was responsible for the laboratory work, including library preparation, target-capture enrichment, and sequencing of some of the samples. N.D., J.v.d.S. and N.J.d.V. carried out the histology of the specimens. J.v.d.S. and N.J.d.V. conducted morphological taxonomic identification and verification of the Red Sea specimens and taxonomic reference material at the Naturalis Biodiversity Center, Leiden, the Netherlands. All authors revised the manuscript.

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Data Availability Statement

The data that support the findings of this study are openly available in HaploscleridMuseomics at https://github.com/PalMuc/HaplosclerIdMuseomics.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Non-collapsed 50% MJR Backbone Phylogram. **Figure S2:** 50% MJR phylograms of Clade E. **Figure S3:** 50% MJR phylograms of Clade C. **Table S1:** Overview of targeted type species of the different (sub)genera. **Table S2:** Samples collected from natural history collections included in this study. **Table S3:** Overview of samples for which we obtained DNA and prepared libraries. **Table S4:** Information on the transcriptome data used in this study. **Table S5:** Sequencing and CSE capture results. **Table S6:** Results of the haplosclerid genera (A) and families (B) hypothesis testing.