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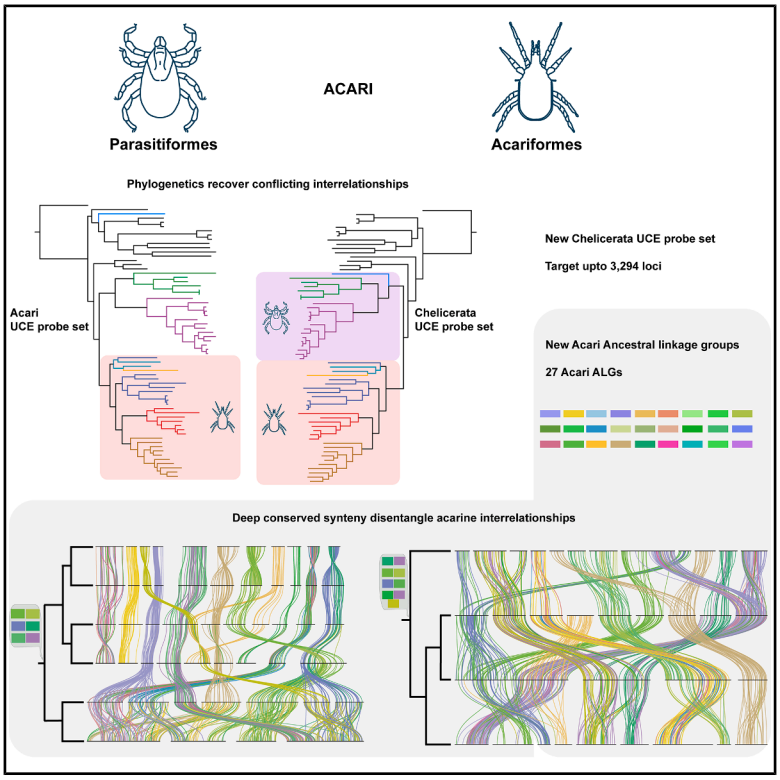
Ancient gene linkages and ultraconserved elements disentangle Acari interrelationships

Graphical abstract



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In brief

Zoology; Entomology; Genomics; Paleogenetics

Highlights

- First Opilioacaridae genome sequenced and Chelicerata-wide UCE set developed
- 90 arachnid genomes interrogated using UCEs resolve Acari interrelationships
- Deep conserved synteny detected in acarine genomes
- Distinct chromosomal fusion patterns in Acariformes versus Parasitiformes

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Ancient gene linkages and ultraconserved elements disentangle Acari interrelationships

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SUMMARY

The interrelationships among Acari groups, Acariformes (mites) and Parasitiformes (ticks and parasitic mites) have been debated for long. We interrogated 90 genomes of Arachnida through a customized Chelicerata-wide ultraconserved elements bait set to resolve higher-level relationships of these two orders, including the first genome of Opilioacaridae, the putative sister group to the remaining Parasitiformes. Datasets with variable locus occupancy thresholds and partitioning schemes supported the monophyly of Acariformes and Parasitiformes, including their major subgroups. Exploration for rare genomic changes as potential character systems was done using a custom 27 Acari-specific ancestral linkage group (AcarLGs). AcarLGs revealed distinct chromosomal fusion patterns within subsets of both orders, indicating robust signal at deeper nodes, supporting their respective monophyly. Syntenic signal is blurred in miniaturized parasitic taxa (e.g., *Tetranychus*, Eriophyidae). We discuss the strong potential of synteny as a phylogenetic character to overcome limitations posed by molecular phylogenies at deeper nodes across karyotypic diversity.

INTRODUCTION

Acariformes and Parasitiformes (ticks and mites) together form a megadiverse group of small-bodied arachnids with over 54,000 described (540 families) and an estimated 1–3 million undiscovered species.[1–4] Over their evolutionary history, they have occupied a broad array of habitats, both terrestrial and aquatic, as well as a large swath of ecological niches, including numerous parasitic lineages.[4–7] Collectively, this pair of taxa comprises the traditional grouping Acari, which is putatively united by the gnathosoma (a feeding apparatus composed of the chelicerae, the ventrally fused palps, and the subcapitulum).[8,9] Acari are part of the arthropod subphylum Chelicerata, which includes several terrestrial taxa (collectively called arachnids) and two extant marine orders, the Xiphosura (horseshoe crabs) and Pycnogonida (sea spiders).[10] The interrelationships among these groups have been tested extensively with morphology, Sanger-sequenced loci, and phylotranscriptomic datasets.[4,8,9,11–18] Despite systemic conflict in phylogenomic datasets, Arachnopulmonata, a group of six orders ancestrally united by paired book lungs, are now an established clade that shares a whole genome duplication.[15–17,19] A series of recent analyses robustly placed the Xiphosura within Arachnida (but outside Arachnopulmonata), rendering Arachnida paraphyletic.[15,16,18] Nevertheless, the placement of the apulmonate groups has remained contentious with conflicting relationships hampered by deep divergence, high diversification rates among some groups, and poor sampling of asymmetrically diverse groups.

Among the apulmonates, Acari remains the least stable group, with most molecular studies not recovering Acari as monophyletic.[2–4,15,17,18] The non-monophyly of Acari is especially supported by molecular studies that have intensively sampled basally branching taxa. It has been shown that exclusion of key sister groups near the base of Parasitiformes tends to result in Acari monophyly, suggesting that Acari is an artifact of taxonomic undersampling.[20] The dogma of Acari monophyly has also been recently challenged by a ground-breaking morphological study, which has shown that the gnathosoma in fact evolved in multiple branches in the Acariformes and Parasitiformes, and is therefore not a synapomorphic trait shared by both groups.[21] Presently, only a pair of methodologically problematic molecular studies still supports Acari monophyly[22,23] (see Ontano et al.[17]; Ballesteros et al, 2022[18]; and Ballesteros et al, 2019[24]). Yet, a robust alternative placement for Acariformes and Parasitiformes has not been obtained. This is because both Parasitiformes and Acariformes, along with Pseudoscorpiones and Palpigradi, are known to exhibit long-branch attraction (LBA) artifacts.[20] These fast-evolving groups cluster together, often at the base of the tree near poorly sampled outgroups, and sequential taxon pruning experiments tend to result in dramatic changes in the phylogenetic placement of these groups, which is symptomatic of LBA. LBA artifacts have been detected both at the level of

ordinal placement within chelicerate phylogeny, as well as for fast-evolving groups within these orders (e.g., Mesostigmata and Eriophyoidea).[3,6,20,25] Previously, secondary structure-aware analyses of nuclear ribosomal genes, a subset of site heterogeneous model (CAT-GTR)-based analyses, and a subset of site recoding (RL2) analyses have all placed Acariformes with solifuges and palpigrades (=Cephalosomata), which share a subdivided prosoma.[2,18] These analyses also recovered solifuges and acariforms (=Poecilophysidea) as a clade (Figure 1A); this pair of orders additionally shares a characteristic sejugal furrow on the ventral prosoma as a potential synapomorphy.[2,14,18]

Interrelationships within Acariformes and Parasitiformes are additionally opaque, owing to a paucity of molecular sequence data for inferring higher-level relationships, as well as limitations in taxonomic sampling. Previous efforts to inferring phylogenetic structure in these groups are based on Sanger-sequenced matrices, which have made considerable headway in identifying deep phylogenetic structures. In Acariformes, Oribatida are understood to be paraphyletic, Endeostigmata is a polyphyletic assemblage, but Trombidiformes and Eriophyoidea are thought to be monophyletic.[3,4,25] Nodal support for these relationships is fairly limited for deep nodes. Parasitiformes, which includes Opilioacariformes, Mesostigmata, Holothyrida, and Ixodida, exhibit a simpler topology, with high nodal support for the reciprocal monophyly of all four groups, although the exact placement of Mesostigmata may be affected by LBA.[6,20]

Advances in genome sequencing across the tree of life have the potential to resolve many of these outstanding questions and offer new data classes for validating phylogenetic hypotheses. For example, genomes offer the prospect of mining for highly informative loci for phylogenetic inference, such as ultraconserved elements (UCEs).[26] Typically obtained using target enrichment through customized lineage-derived bait sets, UCEs have facilitated resolution of various regions of the chelicerate tree of life.[27–35] An Acari probe set was recently developed *in silico* based on available acarine genomes, but recovered highly unexpected relationships in concatenated analyses, such as the derived placement of the orders Opiliones (daddy-longlegs) and Ricinulei (hooded tick spiders) within Parasitiformes.[36] It is possible that this result is attributable to taxonomic undersampling, but the possibility has not been assessed, because UCEs have not been extensively deployed toward resolving mite and tick relationships.

The species richness of Acariformes and Parasitiformes, together with known heterogeneity in evolutionary rates within each of these groups, raises the specter that sequencing-based approaches to resolution may not be sufficient to decipher numerous splits across their phylogeny. A recent exploration of UCE data sufficiency in ants, for example, inferred that hundreds of loci may be necessary to resolve challenging relationships spanned by short internode distances.[37] A separate and independent character system that has begun to revolutionize the resolution of recalcitrant nodes in molecular phylogenies is macrosynteny. It has been shown that homologous genes are frequently retained on the same chromosome for hundreds of millions of years in diverse and highly divergent lineages.[38,39] Physical gene linkages mapping homologous gene sets on chromosomes across deeply divergent lineages have facilitated resolution of highly debated placements, such as the sister group to the remaining Metazoa.[40] The availability of mite and tick genomes offers new avenues to testing acarine relationships using shared linkage groups (LGs), despite the dynamic evolution of genome size. For instance, within Acariformes, mean genome size is below 200 Mb. In contrast, parasitiform genomes are much larger, ranging from 314 Mb to over 2 Gb (Figure 1D).

Here, we explored the analytical power of both UCEs and macrosynteny for resolving acarine relationships. We analyzed a dataset of 90 arachnid genomes, via *in silico* comparison of the Acari-specific UCE probe set[36] to a *de novo* probe set that we developed specific to Chelicerata. As part of this work, we included a *de novo* sequenced low-coverage genome of the early diverging parasitiform group Opilioacaridae. We also explored the signature of higher-level relationships in patterns of macrosynteny as a means of validating phylogenetic inferences based on UCEs. Here, we show that the design and selection of the probe set influence the ability to resolve deep relationships. Separately, we show the signature of rare genomic changes in acarine genomes, which have the potential to resolve definitively contentious relationships that are impacted by LBA.

RESULTS

Raw reads of the *de novo* sequenced *Indiacarus* sp. library on a NovaSeq X sequencer generated 25.4 Gb reads from the library with concentration 1.3 ng/μL and insert size 312. The final assembly using the SPAdes assembler was 2.045 Gbp with N50 1,305 bp and a total of 2,586,046 contigs yielding a coverage of 12.4X.

Chelicerata probe set

We designed a Chelicerata-wide probe set to facilitate capturing more UCE loci from the non-Acari groups that can robustly place Acariformes and Parasitiformes across Arachnida. The probe set was developed using nine chromosomal-level eu-chelicerate genomes representing the orders Scorpiones (scorpions), Pseudoscorpiones (pseudoscorpions), Araneae (spiders), Parasitiformes (ticks), Acariform (mites), Solifugae (camel spiders), Opiliones (harvestmen), and Xiphosura (horseshoe crabs) (Table S1) (STAR Methods). The final probe set after duplicate screening and testing included 36,100 probes targeting 3,294 UCE loci. Average yield for the Acari probe set was 653.5 (n = 65; range = 161–924), whereas that for the Chelicerata probe set was 961 (n = 65; range = 169–2,153). For Acari samples, the average yield for the Acari probe set was 689.5 (n = 39; range = 161–924), whereas that for the Chelicerata probe set was 874.5 (n = 39; range = 169–1,742). Taxon-wise capture efficiency and coverage of both Acari and Chelicerata probe sets are provided in Table S2.

Comparative analysis of UCE datasets

We tested *in silico* both the Acari probe set and our chelicerate probe set on the internal relationships of 29 Acariformes, 21 Parasitiformes, and 15 outgroups. All taxa in this study were drawn from high-quality and highly complete genome projects; to bridge the gap in genomic resources for Opilioacaridae (the putative sister group to Parasitiformes), we sequenced a new

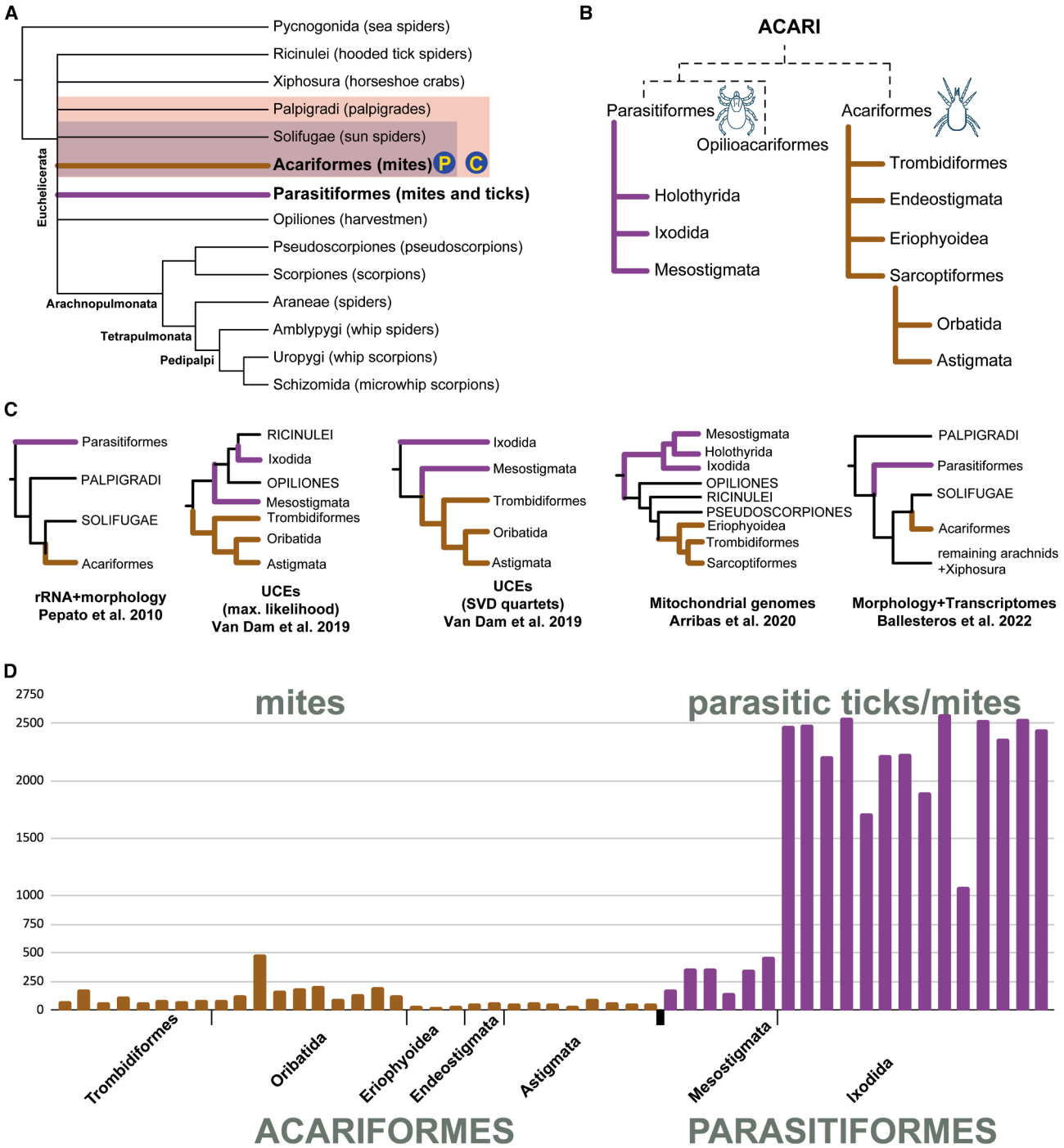

Figure 1. Overview of Acari classification and disparate genome sizes

(A) Acari conflicting relationships including polytomy within Chelicerata with "P" and "C" indicating Poecilophysidea and Cephalosomata groupings, respectively.

(B) Dendrogram of Acari classification.

(C) Alternative hypotheses for the placement of Acari groups within Arachnida.

(D) Genome size distribution across Acari and outgroups.

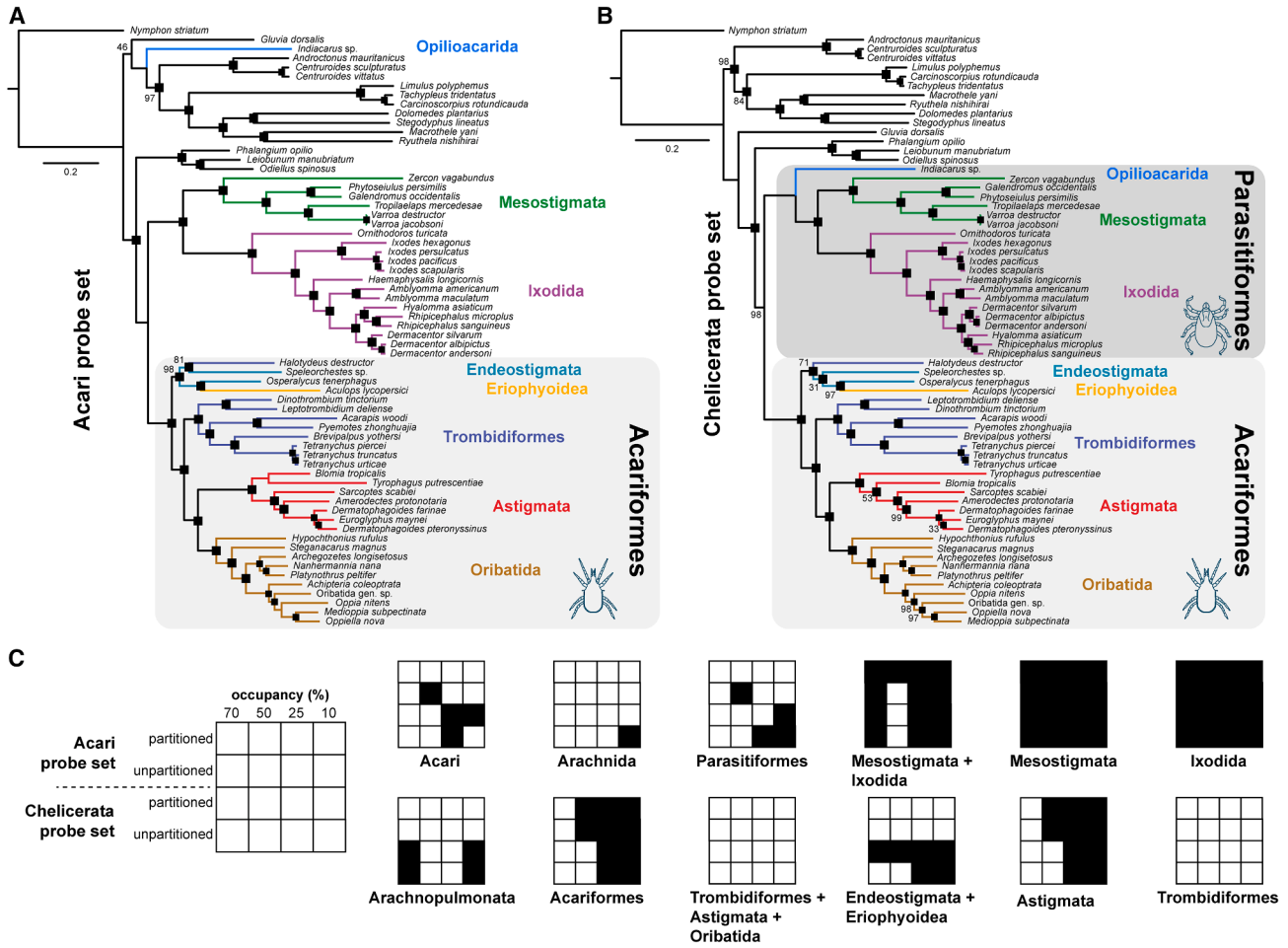

Figure 2. Phylogenetic interrelationships of Acari using ultraconserved elements

(A) Acari probe set (25% occupancy, 783 loci).

(B) Chelicerata probe set (25% occupancy, 955 loci).

(C) Robustness of various clades across a gradient of missing data, Acari and chelicerate probe sets, and data un-/partitioning. Squares at nodes indicate similar nodes in both trees. Nodal support was robust is all nodes (>95%) unless mentioned.

low-coverage genome for an undescribed species of the genus *Indiacarus*. For both probe sets, we designed matrices spanning a family of locus occupancy values (10%, 25%, 50%, and 70%). Locus occupancy thresholds indicate the inclusion of a locus present in that percentage of total taxa. Maximum likelihood inference of each of the eight matrices (36–1,558 loci) with both (1) automated model-fitting of UCE loci and (2) automated model-fitting without partitioning recovered tree topologies with generally high nodal support, measured using ultrafast bootstrapping (Figures 2 and S1). Ixodida and Mesostigmata were stably recovered across all analyses. Due to the phylogenetic placement of *Indiacarus* among the outgroup taxa, Parasitiformes was frequently recovered as diphyletic. Only one partitioned and two unpartitioned analyses from the Chelicerata probe set recovered parasitiform monophyly, whereas the Acari probe set only recovered parasitiforms as a clade in one unpartitioned analyses of a matrix with 50% completeness. Within Parasitiformes, a clade composed of Mesostigmata and Ixodida was recovered in 13 of 16 analyses, with non-monophyly incurred due to the errant placement either of a long-branch Astigmata (*Tyrophagus putrescentiae*) or of *Indiacarus* in a nested position within Parasitiformes.

Acariformes were recovered as monophyletic in six of eight analyses from the Acari probe set, but only four from the Chelicerata probe set, the smallest matrices tending to fail to recover acariform monophyly, as well as most of the higher-level groups within Acariformes. Consistent with densely sampled studies of acariform relationships, we found that a subset of the polyphyletic assemblage Endeostigmata clustered at the base of the acariform tree, albeit with Eriophyoidea as derived within Endeostigmata.[3] The latter is likely an artifact of limited sampling, as eriophyoid placement is known to be unstable across datasets and analytical parameter space.[25] Trombidiformes was consistently recovered as diphyletic owing to the *Halotydeus destructor* either as a sister

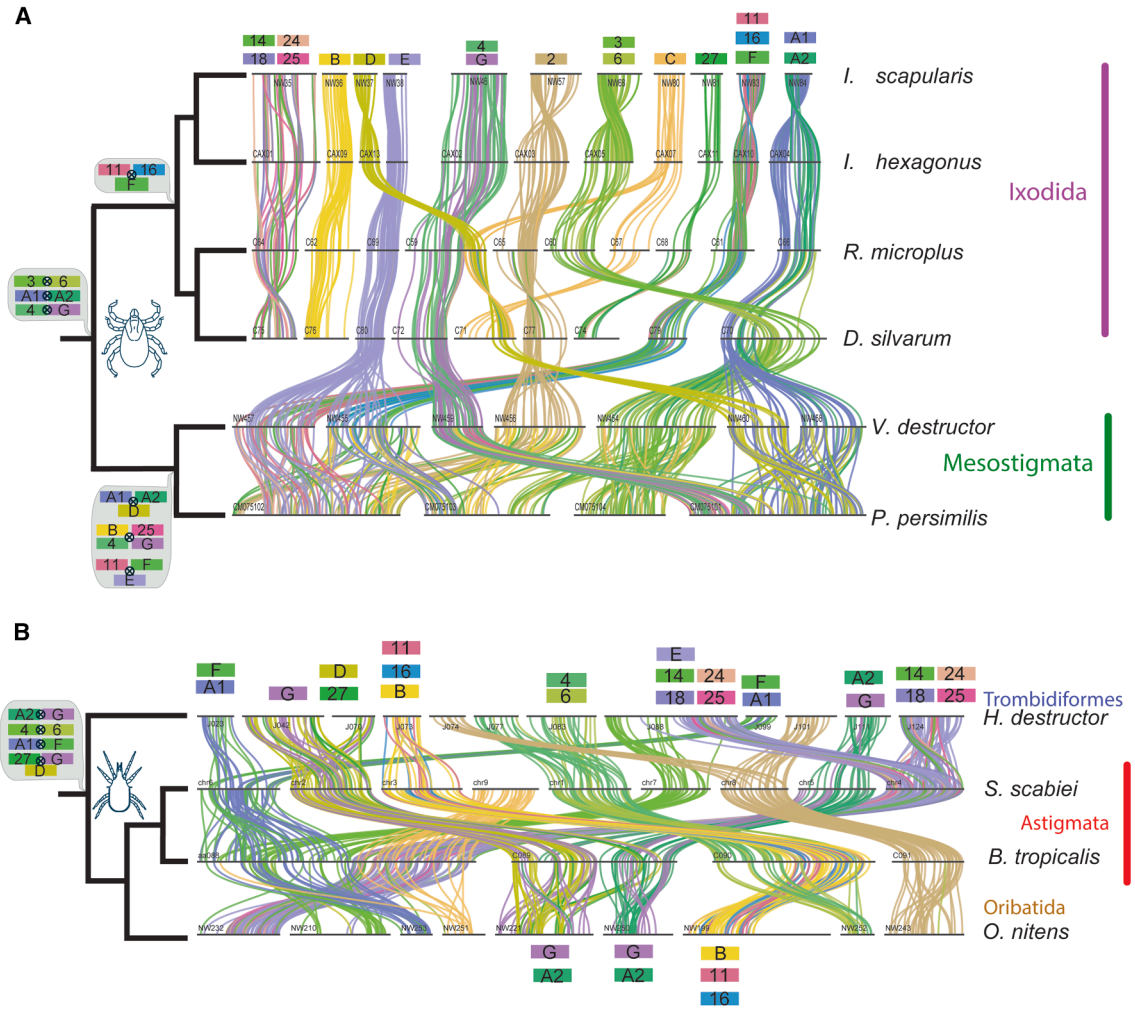

Figure 3. Conserved macrosynteny in Acari genomes using Acari linkage groups (AcarLGs)

(A) Conserved AcarLGs within Parasitiformes.

(B) Conserved AcarLGs within Acariformes.

group to Endeostigmata+Eriophyoidea clade with the some Chelicerata probe set-based matrices or nested within it in the remaining datasets.

Interordinal relationships within Chelicerata were highly unstable and poorly supported. A single unpartitioned analysis recovered arachnid monophyly, without support (86%). Four analyses recovered the monophyly of Arachnopulmonata and four, the monophyly of Acari, without any clear association with analytical criteria (larger versus more complete matrices; partitioned versus unpartitioned analyses).

Macrosynteny

We previously showed that exemplars of both Acariformes and Parasitiformes show strong retention of macrosyntenic signal, when assessed using a universal Myriazoa LG set.[41] To assess the phylogenetic signal available in macrosyntenic patterns, we designed an Acari LG using the chromosomal-level assemblies of *Ixodes scapularis* (Ixodida), *Varroa destructor* (Mesostigmata), and *Sarcoptes scabiei* (Acariformes) as reference genomes. We initially established 27 LGs (Figure S2). Mapping the AcarLG against a non-chelicerate outgroup, the centipede *Strigamia acuminata*, identified an array of phylum-level conserved linkages. We collapsed these plesiomorphic linkages, resulting in 18 LGs (Figure S2). We queried 18 chromosome-level genomes of Acariformes and Parasitiformes for the locations of homologs of these LGs. The list of analyzed chromosome-level genomes is provided in Table S2.

We found that two sets of LGs were consistently fused and mixed across Acariformes, Parasitiformes, Opiliones, and Solifugae: 14 ⊗ 18 ⊗ 24 ⊗ 25 and 11 ⊗ 16. However, in Parasitiformes, LG F was additionally mixed into the latter (11 ⊗ 16 ⊗ F), whereas in Acariformes and Opiliones, LG B was mixed in (11 ⊗ 16 ⊗ B). Parasitiformes share three fusion-and-mixing events: 3 ⊗ 6, A1 ⊗ A2, and 4 ⊗ G (Figures 3 and S3). Extensive merging of the Ixodida

syntenic configuration has occurred in the Mesostigmata genomes, with their smaller genomes and chromosome numbers. Within Ixodida, the genera *Rhipicephalus* and *Dermacentor* are united by the mixing of LGs C ⊗ D, which are separate in *Ixodes*. These non-*Ixodes* taxa also form a clade in our phylogenetic analysis (Figure 2). Acariformes share (4 ⊗ 6), (A1 ⊗ F), (G ⊗ A2), and (27 ⊗ D ⊗ G) (Figure 3). These shared deeply conserved linkages sharing unique fusion with mixing events strongly support a monophyletic Parasitiformes and Acariformes. Their internal groups such as Ixodida, Mesostigmata, and Astigmata are also monophyletic (Figures 3 and S2–S4).

Eriophyoidea is considered a highly derived group, and the only available genome (Eriophyidae sp.; GCA_045522265.1) is 34 Mbp and has only two chromosomes, which do not retain any LGs. Similarly, we did not detect any conserved synteny between the trombidiform *Tetranychus urticae* (three chromosomes) and AcarLGs (Figure S4). *H. destructor*, another trombidiform has retained the AcarLGs shared with the other Acariformes; we surmise that being unscaffolded is not a limitation to incoherence with the *T. urticae* synteny (Figures 3 and S4).

Consistent with recent phylogenomic analyses that have emphasized dense taxonomic sampling,[17,18] we did not recover any evidence of Acari monophyly in our examination of fusion-with-mixing events. Any fusion-with-mixing characters that were common to Acariformes and Parasitiformes were also found in at least one other outgroup taxon (Figures S2–S4).

DISCUSSION

Analytical considerations in the use of ultraconserved element datasets

The increasing use of UCEs in phylogenomics has revolutionized our ability to resolve deep evolutionary relationships across the tree of life, including in arthropods. However, as with any genomic dataset, UCEs present unique analytical challenges that warrant careful scrutiny, particularly in groups with complex histories such as the Acari (mites and ticks). We provide a critical evaluation of UCE probe performance for resolving Acari interrelationships, comparing the previously published Acari probe set with the newly developed, broader Chelicerata-focused probe set. These results yield important insights into both the utility and the limitations of UCEs for resolving Acari relationships.

The Acari probe set in its original study[36] produced unexpected topologies within Acari, notably recovering Parasitiformes as non-monophyletic. Such a result was at odds with past morphological and molecular phylogenetic inferences, likely owing to taxon sampling. Our analyses across a broader taxonomic sampling reveal that many of these anomalies may stem from missing data or locus dropout, especially in undersampled lineages. For instance, Opilioacariformes—a key parasitiform lineage—was inconsistently recovered within Parasitiformes when using the Acari probe set. This inconsistency likely reflects a combination of sparse UCE recovery for certain terminals (e.g., *Indiacarus*; see Table S2) and probe bias toward more derived lineages. This is evident from higher locus recovery by the Acari probe set in some derived taxa such as Astigmata and Mesostigmata. However, the pattern is reversed in all other Acari groups, including Opilioacaridae and the outgroups (Table S2). The newer Chelicerata-wide probe set, by contrast, exhibited more consistent placement of Opilioacariformes in the low-occupancy matrices, suggesting improved performance in recovering deeper splits. Lower-occupancy datasets include higher number of loci facilitating more informative loci, however, at the cost of some missing loci for some taxa. This trend of robustly supported congruent deeper relationships with low-occupancy UCE datasets rendering more loci concurs with that of ants, spiders, solifuges, etc.[29,31,42]).

Overall, we found high congruence between the two probe sets at shallower to intermediate nodes within both Acariformes and Parasitiformes, indicating that UCEs are generally robust for resolving mid-level relationships within Acari. However, more pronounced differences between probe sets emerged when assessing deeper divergences, particularly among chelicerate orders. These differences likely reflect systematic biases in UCEs for resolving ancient divergences, including substitutional saturation and disparate genome sizes, which may obscure signal at these depths. Notably, neither probe set consistently recovered Arachnopulmonata or Parasitiformes as monophyletic—groups that have received varying support across different genomic and transcriptomic studies. In the case of Arachnopulmonata, only the Chelicerata probe set, which includes more broadly conserved loci across deep chelicerate lineages, recovered it as monophyletic in any analyses, highlighting the need for careful locus selection when addressing ancient relationships.

The recovery of Acari as monophyletic in some analyses should be interpreted cautiously. Although this result may appear to lend support to a long-debated hypothesis, several factors limit our ability to test Acari monophyly rigorously. First, potential LBA artifacts—particularly affecting highly divergent acariform and parasitiform lineages—were not explicitly modeled or corrected for in our study, as our previous works have shown that depth of taxonomic sampling outperforms model-based approaches to suppressing LBA.[17] Second, our genome-only dataset lacks several key outgroup taxa (e.g., Ricinulei and Palpigradi), which are critical for polarizing chelicerate relationships and assessing whether Acari represent a true clade or an assemblage of long-diverged lineages. Without these taxa, topological results regarding Acari monophyly remain equivocal and potentially artifactual. The lack of Ricinulei and Palpigradi genome assemblies is peremptory to resolving the euchelicerate polytomy.[43]

Macrosynteny as a phylogenetic character for resolving acarine interrelationships

The use of macrosynteny as a phylogenetic character is emerging as a powerful complement to sequence-based phylogenomics, particularly in lineages where high rates of molecular evolution or gene loss obscure deep evolutionary signals. Our findings underscore the value of macrosyntenic patterns as phylogenetically informative features in Acariformes and Parasitiformes—an extremely diverse assemblage with several morphologically reductive lineages. Despite notable differences in genome sizes, we recovered distinct, conserved macrosyntenic signals within both acariform lineages and parasitiform lineages, suggesting that genome architecture has remained relatively stable since

their divergence. These patterns not only reinforce the monophyly of these two clades but also provide a structural basis for resolving more recalcitrant branches of the acariform and parasitiform trees.

The monophyly of Acari has long been debated, with the gnathosoma historically considered as a solid character uniting them, although its common origin has now been overturned by morphological study in tandem with intensive sampling of both groups.[21] Consistent with Bolton's assessment, we did not find any macrosyntenic mixing characters that supported the monophyly of Acari, even with a limited sampling of outgroups; the absence of evidence supporting Acari is congruent with molecular phylogenetic studies that have supported alternative relationships, such as Poecilophysidea (Acariformes + Solifugae) and Cryptognomae (Ricinulei + Parasitiformes).[2,18]

The application of these techniques to the remaining arachnid orders awaits the generation of new genomic resources for those groups. In particular, sequencing of a high-quality Ricinulei genome is necessary to test competing hypotheses of acarine relationships. Our macrosynteny analyses inferred a shared (11 ⊗ 16) character, which is potentially informative for apulmonate relationships. The informativeness of this character can be tested in future analyses, as genomes become available for understudied groups like Ricinulei and Palpigradi. A key taxon in this context is Opilioacaridae. A chromosome-level opilioacarid genome will be instrumental in definitively resolving its placement, specifically, the presence or absence of three conserved chromosomal fusion-and-mixing events that define Parasitiformes: (3 ⊗ 6), (A1 ⊗ A2), and (4 ⊗ G). If these syntenic fusions are also found in Opilioacaridae, it would provide compelling genomic evidence for its placement within Parasitiformes and help resolve long-standing ambiguities in acariform-parasitiform relationships.

Conclusion

In this study, we demonstrate that conserved gene linkages and their arrangements and phylogenetic inference elucidate the systematic groupings within Acariformes and Parasitiformes. We establish a framework to decipher the polarity of phenotypic traits within Acari. More genomic resources for groups such as Holothyrida, Eriophyoidea, Endeostigmata, and particularly the early diverging Opilioacarida are required to test the evolutionary history of these enigmatic groups. This information will form an additional piece of evidence in the run to resolve the notorious apulmonate polytomy in the arachnid tree of life.

Limitations of the study

While we observe strong macrosyntenic conservation within sampled lineages, the absence of genomic data from several key taxa limits our ability to fully assess the extent and phylogenetic relevance of these patterns. Notably, Trombidiformes—an extremely diverse and morphologically variable group within Acariformes—remains underrepresented in genome-scale analyses. Similarly, Endeostigmata, which our results retain as paraphyletic, requires broader sampling to clarify whether this paraphyly reflects true evolutionary history or is an artifact of incomplete lineage sorting or data resolution.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to the lead contact, Siddharth Kulkarni (siddharth.ccmb@csir.res.in).

Materials availability

This study did not generate new unique materials.

Data and code availability

- Data: The *Indiacarus* sp. (Opilioacaridae) low-coverage genome is available under the NCBI Sequence Read Archive (SRA) accession number SRR27200377 under BioProject PRJNA1298856. All the other data underlying this article are made available on Mendeley link (https://doi.org/10.17632/k3mp2p6g23.1).
- Code: No new code was generated in this study. Direct links to used software in this study are provided in the key resources table.
- All other items: Any additional information required to reanalyze the data reported in this paper are available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, S.K.; methodology, J.P.B., R.M., N.S., P.P.S., and S.K.; resources, S.K. and P.P.S.; writing – original draft, S.K. and P.P.S.; writing – review & editing, P.P.S. and S.K.; supervision, S.K.; project administration, S.K.; funding acquisition, S.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Custom probe set design
  - Acari linkage group design
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Data matrices were deposited on Mendeley	This study	http://www.doi.org/10.17632/k3mp2p6g23.1
Raw reads are available from the NCBI	This study	PRJNA1298856
Software and algorithms		
IQ-TREE2	Nguyen et al.[44]	http://www.iqtree.org/
PHYLUCE pipeline	Faircloth[45]	https://phyluce.readthedocs.io/en/latest/
ODP	Schultz et al.[40]	https://github.com/conchoecia/odp
BRAKER3	Gabriel et al.[46]	https://github.com/Gaius-Augustus/BRAKER
RepeatModeler2	Flynn et al.[47]	https://github.com/Dfam-consortium/RepeatModeler
Other		
Chelicerata probe set	This study	http://www.doi.org/10.17632/k3mp2p6g23.1
Acari probe set	Van Dam et al.[36]	https://doi.org/10.1111/1755-0998.12962

Method details

Opilioacarida genome

DNA was extracted from eight legs of a single individual of *Indiacarus* sp., using QIAmp DNA extraction kit (Qiagen#56504). The library preparation was carried out using Ultra II DNA lib preparation for Illumina Kit (NEBNext #E7645S/L). Final library was quantified using Qubit 4.0 fluorometer (Thermofisher #Q33238) using DNA HS assay kit (Thermofisher #Q32851) following manufacturer's protocol. To identify the insert size of the library, we queried it on Tapestation 4150 (Agilent) utilizing high sensitive D1000 screentapes (Agilent # 5067- 5582) following manufacturers' protocol. Sequencing was done on a NovaSeq X machine with paired end 150 bp reads.

Species sampling for designing the Chelicerata probe set included seven chromosomal genomes comprising one chelicerate Order each (Table S1). Sampling for phylogenetic analysis included 90 genomes using three Opiliones, four Araneae, three Xiphosura, three Scorpiones, one Solifugae and Pycnogonida (sea spiders) as outgroups with the pycnogonid as root. For macrosynteny analyses, Acari specific linkage group was designed using three taxa, an Ixodida: *Ixodes scapularis*, a Mesostigmata: *Varroa destructor* and an acariform *Sarcoptes scabiei* (NCBI Genome accession numbers in Table S2).

Custom probe set design

We developed a Chelicerata probe set using available chromosomal scaffolded genomes representing seven orders. We used PHYLUCE version 1.7.1[26,45] to design the probe set, following the tutorial on https://phyluce.readthedocs.io/en/latest/tutorials/tutorial-4.html. The downloaded .fna genomes listed in Table S1, were converted to FASTA format and file headers were modified for compatibility with the PHYLUCE pipeline using SeqIO, available on Bioconda.[48] Copies of the genomes in 2bit format, required further downstream in the pipeline, were created using faToTwoBit.[49] ART[50] was then used to simulate 100-bp short reads with 200-bp insert size (150 standard deviation) from all exemplar genomes covering the base genome approximately 2X. We used the harvestman assembly of *Odiellus spinosus* (GCA_963920705.1) as our base genome. The simulated reads were individually aligned to the base genome using STAMPY,[51] identifying putatively conserved loci with less than 5% sequence divergence to the base genome. Unmapped reads were removed from the alignment files using the view function in SAMtools,[52] and the cleaned alignments were converted to BED format, sorted by position along scaffolds, and proximate contigs were merged using BEDTools.[53] Finally, phyluce_probe_strip_masked_loci_from_set in PHYLUCE was used to filter out putatively conserved loci that are repetitive regions, by removing all contigs shorter than 80 bp and where more than 25% of the base genome were masked.

An SQLite database was created to query conserved loci shared across several taxa using these pairwise alignment files. From this database, we identified those loci shared between the base genome and three out of six exemplar genomes, and extracted sequences corresponding to these loci from the base genome, buffered to 160 bp, with phyluce_probe_get_genome_sequences_from_bed. For each of these sequences, two 120-bp probes with 40 bp overlap were designed to achieve 3X tiling density at the center of the conserved locus. Potentially problematic probes with more than 25% masked bases, below 30% or above 70% GC content, as well as potential duplicate probes with more than 50% coverage and identity were subsequently removed, to create a temporary probe set.

To design the final probe set, the temporary probes were aligned back to all exemplar genomes, now also including, a Xiphosura, *Tachypleus tridentatus* (GCA_015741085.1), and a spider, *Macrothele cretica* (GCA_964417605.1) with a minimum identity of 50%.

Sequences of 180 bp length were extracted from the targeted conserved loci in all taxa and written to FASTA files. Another SQLite database with the matches of the temporary probes to conserved loci across all taxa was created and used to identify loci that were recovered by a temporary probe in at least four out of the nine taxa (8 exemplar, 1 base genome). For each of these loci, two 120-bp probes with 3X tiling density were designed from all taxa, filtered and duplicates removed as described above. In-silico tests of the UCE probe set were performed by assessing locus recovery across a range of chelicerate and outgroup genomes, using phyluce_assembly_match_contigs_to_probes after aligning the probe set to these genomes (see Table S1).

We analyzed a total of NCBI data sets including 90 chelicerate genomes. We extracted UCEs from these by firstly, converting from fasta to 2bit format using FaToTwoBit (http://hgdownload.soe.ucsc.edu/admin/exe/). Then, we created a temporary relational database to summarize probe to assembly match using: phyluce_probe_run_- multiple_lastzs_sqlite function on the 2-bit files.The ultraconserved loci were recovered by the phyluce_probe_slice_sequence_from_genomes command. The resulting FASTA files were treated as contigs and used to match the reads to the Chelicerata probe set. Then, each of these 90 genomes was aligned with the probe, where we screened for orthologous and duplicate loci with minimum identity and minimum coverage of 80 and 80 matched to the Chelicerata probe set, as suggested by Bossert and Danforth.[54] We utilized the PHYLUCE tool phyluce_align_get_gblocks_trimmed_alignments_from_untrimmed to generate Gblocks files.

Model selection for the unpartitioned analyses was allowed using the TEST function and for the partitioned analyses for each locus using the MFP + MERGE flag via ModelFinderPlus.[55] We applied locus occupancies of 0.1, 0.25, 0.5 and 0.7 to all data sets to assess their effect on tree topology. Model selection was allowed for each data set using the TEST function.[55,56]

Acari linkage group design

For comparative analyses of macrosynteny, genomic positions of orthologs, we developed an ancestral linkage group with the three Acari representatives using a three-way orthology search by finding reciprocal-best protein matches using diamond[57] and OrthoFinder v.2.3.7[58] implemented in odp v.0.3.0.[40] The resulting 27 linkage groups were mapped against an outgroup- *Strigamia acuminata* (Myriapoda). The resulting ALG was then used to identify macrosyntenic chromosomes between various chelicerata genomes by performing Bonferroni-corrected one-sided Fisher's exact tests.[38] We used in-built tools in odp v.0.3.0 to visualize syntenic patterns as Oxford dot plots and ribbon plots. Annotation for genomic rearrangements follow Lewin et al.[59]: a tensor for fusion with mixing ($\otimes$).

QUANTIFICATION AND STATISTICAL ANALYSIS

All parameters are reported either in individual figures or corresponding figure legends where relevant. Parameters used in the analyses can be found in method details. Please refer to key resources table for direct links to the softwares used in this study.