Spotlight Article

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The Phylogeny and Evolution of the Flashiest of the Armored Harvestmen (Arachnida: Opiliones)

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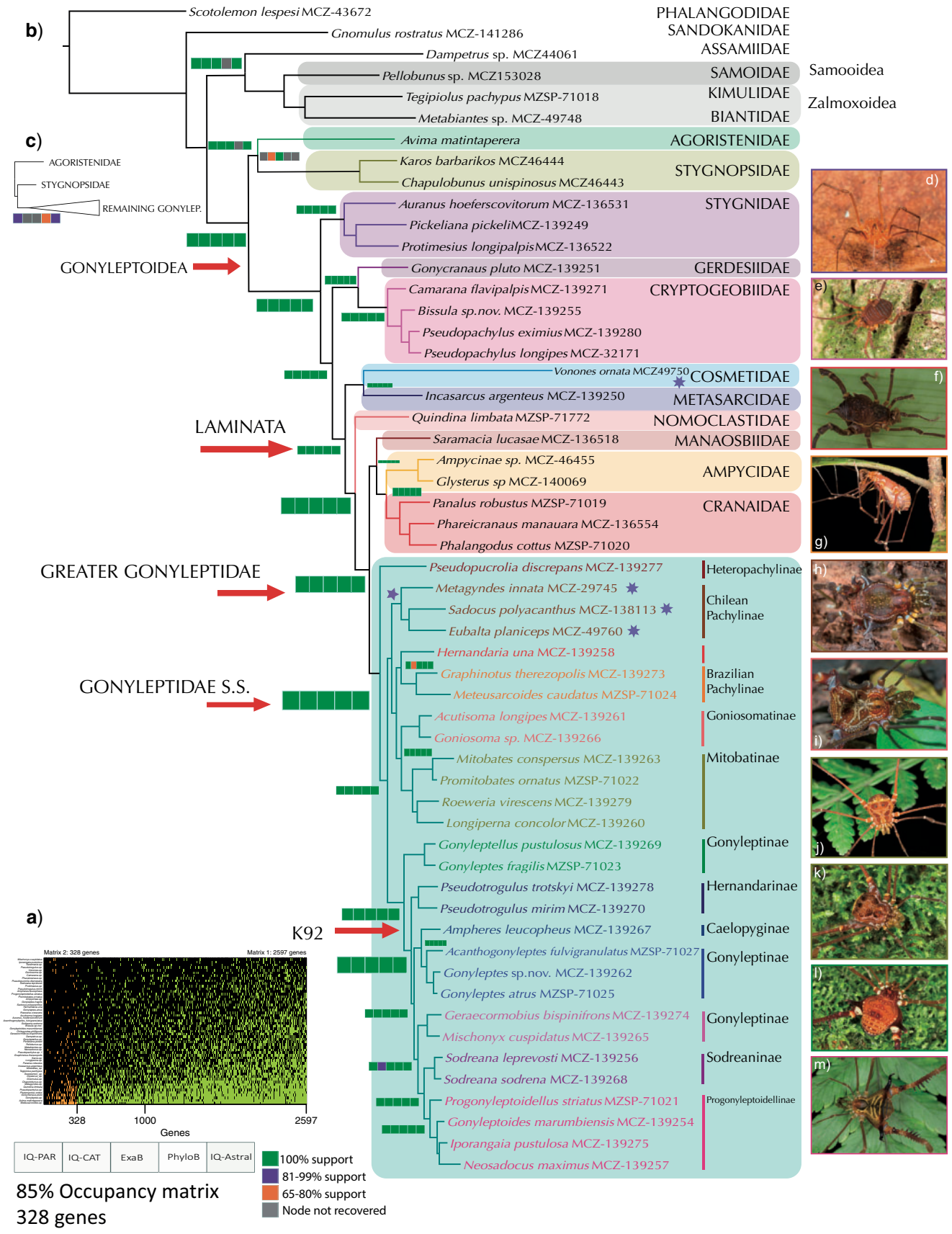
Abstract.—Gonyleptoidea, largely restricted to the Neotropics, constitutes the most diverse superfamily of Opiliones and includes the largest and flashiest representatives of this arachnid order. However, the relationships among its main lineages (families and subfamilies) and the timing of their origin are not sufficiently understood to explain how this tropical clade has been able to colonize the temperate zone. Here, we used transcriptomics and divergence time dating to investigate the phylogeny of Gonyleptoidea. Our results support the monophyly of Gonyleptoidea and all of its families with more than one species represented. Resolution within Gonyleptidae s.s. is achieved for many clades, but some subfamilies are not monophyletic (Gonyleptinae, Mitobatinae, and Pachylinae), requiring taxonomic revision. Our data show evidence for one colonization of today's temperate zone early in the history of Gonyleptidae, during the Paleogene, at a time when the Neotropical area extended poleward into regions now considered temperate. This provides a possible mechanism for the colonization of the extratropics by a tropical group following the Paleocene–Eocene Thermal Maximum, explaining how latitudinal diversity gradients can be established. Taxonomic acts: Ampycidae Kury 2003 is newly ranked as family; *Neosadocus* Mello-Leitão is transferred to Progonyleptoidellinae (new subfamilial assignment). [Arachnids; biogeography; phylogenomics; transcriptomics.]

Some of the most spectacular harvestmen are those of the Neotropical superfamily Gonyleptoidea, which display an amazing variation in size, shape, and color (Fig. 1). Gonyleptoidea comprises 2039 described species (Kury 2000–2020) and includes two of the most diverse families of Opiliones, Cosmetidae (>710 spp.), and Gonyleptidae (>820 spp.). Most gonyleptoids are restricted to humid mountain ranges or tropical lowlands of the Neotropical region with less than 1% of its diversity present at higher latitudes, in southern North America (7 spp. of Cosmetidae and 10 spp. of Stygnopsidae) and southern Patagonia (ca. 90 spp. of Gonyleptidae). However, the systematics of Gonyleptoidea at the family and subfamily levels has changed drastically through the years, hence requiring considerable revision before attempting to understand the colonization of the temperate zones by a group composed largely of tropical species.

During most of the 20th century, the classification of harvestmen relied on a system in which a limited number of characters were used to diagnose families and subfamilies. Higher-level Opiliones systematics has flourished since the early days of morphological cladistic analyses (Martens 1986; Kury 1993; Shultz 1998; Giribet et al. 1999; Giribet et al. 2002), resulting in numerous DNA sequence-based data sets published (e.g., Giribet et al. 1999, 2002, 2010; Shultz and Regier 2001; Pinto-da-Rocha et al. 2014; Cruz-López et al. 2016; Wong et al. 2017), including recent phylogenomic treatments (Hedin et al. 2012; Sharma and Giribet 2014; Fernández et al. 2017; Aharon et al. 2019). However, the relationships among the gonyleptoid families have received little attention using this new generation of data, despite having a long history of systematic work using mostly morphology (e.g., Kury 1994; Pinto-da-Rocha 2002; Kury and Villarreal 2015). In parallel, molecular studies have investigated questions around Gonyleptoidea using Sanger-based approaches (Giribet et al. 2010; Sharma and Giribet 2011; Garwood et al. 2014; Cruz-López et al. 2016; Wong et al. 2017) or transcriptomes (Fernández et al. 2017). Only a few morphological and Sanger-based molecular analyses have examined relationships within Gonyleptoidea and related families (Kury 2014; Pinto-da-Rocha et al. 2014; Bragagnolo et al. 2015; Kury and Villarreal 2015) leading to an unstable system and to a proliferation of hypotheses of relationships.

The latitudinal diversity gradient (LDG), a decrease in the richness of species from the equator to the poles (Fischer 1960), has often been explained with two models, whether the tropics act as a *cradle* (generating more lineages than higher latitudes) or as a *museum* (preserving more lineages than higher latitudes, i.e., they have less extinction), thus balancing speciation and extinction. Yet, a third model, the "out of the tropics" (OTT) model seems to better explain LDGs at least in marine bivalves (Jablonski et al. 2006) and mammals (Rolland et al. 2014). The OTT model posits that lineages not only preferentially originate (cradle) in the tropics but also persist there (museum) and expand poleward. Few studies have tested aspects of the LDGs using evolutionary processes, as nearly complete phylogenies are needed, as in the case of a study focusing on mammals (Rolland et al. 2014). It is thus our aim to leverage the power of transcriptomics to explore how the tropical Gonyleptoidea has a few lineages currently



inhabiting the temperate zone and explain how LDGs may have been established in this large arthropod clade.

Materials and Methods

Taxon Selection

Our phylogenomic study comprises 55 taxa, 49 of them representing all families of Gonyleptoidea with the exception of the recently described family Otilioleptidae—based on a single troglomorphic species from Argentina (Acosta 2019)—and most subfamilies of Gonyleptidae, with four exceptions: Cobaninae, Bourguyiinae, Gonyassamiinae, and the monotypic Pachylospeleinae. The outgroups include six taxa in other Laniatores families within the subclade Grassatores. Information about sampling localities can be found in the online MCZ database collection (http://mczbase.mcz.harvard.edu) and in the Supplementary Table S1 available on Dryad at https://doi.org/10.5061/dryad.d2547d81.

Wet Lab Protocols

All tissues were collected fresh, preserved in RNA*later* (Ambion) and stored at −80°C. RNA laboratory procedures, library construction, and sequencing, closely followed the methods described in our previous research (Fernández et al. 2017; Benavides et al. 2019). We isolated total RNA using TRIzol reagent (Invitrogen) and purified mRNA with Dynabeads (Invitrogen). We assessed the quantity and quality of mRNA with the 2100 Bioanalyzer (Agilent technologies). For cDNA library construction, we used the PrepX RNA-Seq Library kit and the Apollo 324 System (Wafergen), assessing the success of library construction following 8–15X Polymerase Chain Reaction (PCR) amplification by measuring DNA concentration with qPCR (Kapa Library Quant Kit) and library fragment size distribution with the 4200 TapeStation. We pooled up libraries together at equimolar concentrations, confirmed pool concentration with TapeStation and qPCR and sequenced final pools on the Illumina HiSeq 2500 platform with paired ends of 150 bp at the Bauer Core Facility at Harvard University. New sequence reads are deposited in the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA, BioProject PRJNA556673, SAMN12670385-SAMN12670426, SAMN14113753); library indexes and assembly statistics can be found in Table 1.

Sanitation, Assembly, and Identification of Coding Regions

The sequencing facility demultiplexed and converted raw data from BCL to FASTQ. We performed correction of random sequencing errors in our Illumina RNA-seq FASTQ files with Rcorrector (Song and Florea 2015) and quality trimming to remove adapters and low-quality reads (shorter than 50bp) with TrimGalore! v0.5.0 (Krueger 2018). We filtered out rRNA and mtDNA with Bowtie2 v2.2.9, using custom databases built from all relevant rRNA and mtDNA sequences available from GenBank, and assembled reads de novo with Trinity (Grabherr et al. 2011; Haas et al. 2013). We executed a second Bowtie2 run on the assemblies and reduced sequence redundancy with CD-HIT v4.6.4 (Li and Godzik 2006; Fu et al. 2012) by eliminating transcripts with sequence identity >95%; we used Transdecoder v3.0 (Haas et al. 2013) to translate transcripts into amino acids and a custom python script (*choose_longest_iso.py*) to select the longest isoform of each gene. Completeness of our assemblies was assessed with BUSCO (Simão et al. 2015). The assessment tool finds matches to sets of genes that are expected to be present as single-copy orthologs in a given taxon when compared with the Metazoan Database.

Orthology assignment and matrix construction

Orthology assignment for our samples was carried out with the Orthologous Matrix Algorithm (OMA; Altenhoff et al. 2011; Altenhoff et al. 2019); alignment of each individual orthogroup was performed with MAFFT v7.309 with the -auto option (Katoh and Standley 2013), and positions with more than 80% missing data were removed with the script *trimEnds.sh* (Cunha and Giribet 2019).

For our phylogenetic analyses, we assembled three matrices using gene occupancy and extreme evolutionary rates thresholds. For Matrices 1 and 2 (gene occupancy matrices), we targeted a minimum gene occupancy of 50% and 85% of the orthogroups using

Figure 1. a) Occupancy matrices used to infer gonyleptoid evolutionary relationships. Matrix 1 (green + orange) with 2597 genes is the largest (50% occupancy), while Matrix 2 (orange) is the smallest, with the best sampled 328 orthogroups (85% occupancy); b) Gonyleptoid relationships inferred from the analyses of Matrix 2, with a nonpartitioned analysis and model search under maximum likelihood (model LG +C60; ln$L$ = −941,482.997). c) Alternative hypothesis for gonyleptoid relationships. d)–m) Live habitus of representatives of Gonyleptoidea: d) *Auranus hoeferscovitorum* MCZ-136531 (Stygnidae); e) *Pseudopachylus longipes* (Cryptogeobiidae); f) *Glysterus* sp. MCZ-140069 (Ampycidae); g) *Phareicranaus manauara* (Cranaidae); h) *Eubalta planiceps* MCZ-49760 (Gonyleptidae, Pachylinae); i) *Acutisoma longipes* MCZ-139261 (Gonyleptidae, Goniosomatinae); j) *Promitobates ornatus* MZSP-71022 (Gonyleptidae, Mitobatinae); k) *Longiperna concolor* MCZ-139260 (Gonyleptidae, Mitobatinae); l) *Gonyleptes fragilis* MZSP-71023 (Gonyleptidae, Gonyleptinae); m) *Progonyleptoidellus striatus* MZSP-71021 (Gonyleptidae, Progonyleptoidellinae). Support values depicted in each node refer to IQ-TREE with gene-partitioned and model search including LG4 mixture model and accounting for heterotachy (IQ-PAR), IQ-TREE nonpartitioned analysis with CAT-model equivalent (IQ-CAT), ExaBayes (ExaB), PhyloBayes (PhyloB) and a coalescent-based approach with Astral (IQ-Astral), for Matrix 2 (85% occupancy, 328 genes). The clade of the Chilean Pachylinae is indicated with a purple star; purple stars on terminals indicate temperate species. See Materials and methods for details (online version in color).

TABLE 1. Assembly statistics for the new Illumina libraries of 42 Opiliones of the superfamily Gonyleptoidea.

Family/subfamily	Species	MCZ accession	Source	Paired reads	Adaptor	N50	Final number peptides	Bioproject	SRA accesion
Agoristenidae	*Avima matintaperera*	MCZ-136560	Fernández et al.	5,096,624		394	9667		SRR5241472
Stygnopsidae	*Chapulobunus unispinosus*	MCZ-46443	Fernández et al.	26,199,967		564	18,608		SRR5241476
	Karos barbarikos	MCZ-46444	This article	32,598,640		593	25,080		SAMN14113753
Stygnidae	*Auranus hoeferscovitorum*	MCZ-136531	This article	64,496,194	TTAGGC	555	23,837	PRJNA556673	SAMN12670389
	Pickeliana pickeli	MCZ-139249	This article	16,275,828	TGACCA	454	17,446	PRJNA556673	SAMN12670413
	Protimesius longipalpis	MCZ-136522	Fernández et al.	10,706,434		929	19,892		
Gerdesiidae	*Gonycranaus pluto*	MCZ-139251	This article	47,234,889	GCCAAT	328	6026	PRJNA556673	SAMN12670396
Cryptogeobiidae	*Bissula* sp.nov.	MCZ-139255	This article	16,431,417	ACTTGA	388	18,865	PRJNA556673	SAMN12670390
	Camarana flavipalpi	MCZ-139271	This article	56,848,236	GATCAG	484	24,457	PRJNA556673	SAMN12670391
	Pseudopachylus longipes	MCZ-32171	Fernández et al.	9,548,077		522	14,427		SRR5241471
	Pseudopachylus eximius	MCZ-139280	This article	17,533,612	TAGCTT	417	19,252	PRJNA556673	SAMN12670416
Cosmetidae	*Vonones ornata*	MCZ-49750	Fernández et al.	66,096,788			28,390		SRR1145738
Metasarcidae	*Incasarcus argenteus*	MCZ-139250	This article	35,918,463	ACAGTG	422	14,062	PRJNA556673	SAMN12670404
Nomoclastidae	*Quindina limbata*	MZSP-71772	This article	16,309,674	TTAGGC	350	7450	PRJNA556673	SAMN12670420
Ampycidae	Ampycinae sp.	MCZ-46455	This article	38,608,253	CAGATC	508	22,037	PRJNA556673	SAMN12670388
	Glysterus sp.	MCZ-140069	This article	17,154,402	CGATGT	469	20,047	PRJNA556673	SAMN12670394
Manaosbiidae	*Saramacia lucasae*	MCZ-136518	Fernández et al.	32,068,970		596	26,362		SRR5241473
cranaidae	*Panalus robustus*	MZSP-71019	This article	43,122,152	CGATGT	363	13,107	PRJNA556673	SAMN12670411
	Phalangodus cottus	MZSP-71020	This article	37,207,972	ATCACG	410	7411	PRJNA556673	SAMN12670412
	Phareicranaus manauara	MCZ-136554	Fernández et al.	18,556,469		763	21,521		SRR5241475
Gonyleptidae									
Heteropachylinae	*Pseudopucrolia discrepans*	MCZ-139277	This article	17,167,356	TGACCA	538	19,952	PRJNA556673	SAMN12670417
Hernandariinae	*Hernandaria una*	MCZ-139258	This article	17,641,741	GGCTAC	440	18,000	PRJNA556673	SAMN12670403
	Pseudotrogulus trostkyi	MCZ-139278	This article	46,512,679	ACAGTG	538	22,971	PRJNA556673	SAMN12670419
	Pseudotrogulus mirim	MCZ-139270	This article	34,334,557	ACTTGA	562	22,244	PRJNA556673	SAMN12670418
Pachylinae	*Metagyndes innata*	MCZ-29745	Fernández et al.	6,966,617		472	21,678		SRR5241474
	Eubalta planiceps	MCZ-49760	This article	54,025,775	CGATGT	424	18,912	PRJNA556673	SAMN12670392
	Sadocus polyacanthus	MCZ-138113	This article	22,417,530	ACAGTG	514	24,234	PRJNA556673	SAMN12670422
	Graphinotus therezopolis	MCZ-139273	This article	15,793,752	TAGCTT	386	12,426	PRJNA556673	SAMN12670402
	Meteusarcoides caudatus	MZSP-71024	This article	24,030,916	CGATGT	274	2633	PRJNA556673	SAMN12670407

(*Continued*)

TABLE 1. Continued

Family/subfamily	Species	MCZ accession	Source	Paired reads	Adaptor	N50	Final number peptides	Bioproject	SRA accesion
Goniosomatinae	*Acutisoma longipes*	MCZ-139261	This article	59,499,983	GGCTAC	476	18,999	PRJNA556673	SAMN12670386
	Goniosoma sp.	MCZ-139266	This article	23,037,622	TTAGGC	573	22,729	PRJNA556673	SAMN12670395
Mitobatinae	*Longiperna concolor*	MCZ-139260	This article	19,462,927	TAGCTT	458	16,601	PRJNA556673	SAMN12670406
	Mitobates conspersus	MCZ-139263	This article	42,805,149	CAGATC	406	20,298	PRJNA556673	SAMN12670409
	Promitobates ornatus	MZSP-71022	This article	33,733,216	TGACCA	404	18,009	PRJNA556673	SAMN12670415
Roeweriinae	*Roeweria virescens*	MCZ-139279	This article	54,511,076	TTAGGC	424	21,812	PRJNA556673	SAMN12670421
Caelopyginae	*Ampheres leucopheus*	MCZ-139267	This article	24,827,951	ACAGTG	495	19,004	PRJNA556673	SAMN12670387
Gonyleptinae	*Gonyleptellus pustulosus*	MCZ-139269	This article	61,000,382	ACTTGA	387	19,189	PRJNA556673	SAMN12670397
	Gonyleptes fragilis	MZSP-71023	This article	36,166,788	CGATGT	414	17,897	PRJNA556673	SAMN12670399
	Acanthogonyleptes fulvigranulatus	MZSP-71027	This article	47,901,063	GATCAG	327	17,633	PRJNA556673	SAMN12670385
	Gonyleptes atrus	MZSP-71025	This article	19,851,432	ACTTGA	436	16,164	PRJNA556673	SAMN12670398
	Gonyleptes sp.	MCZ-139262	This article	16,097,139	TAGCTT	388	9125	PRJNA556673	SAMN12670400
	Geraecormobius bispinifrons	MCZ-139274	This article	17,603,606	GGCTAC	412	11,430	PRJNA556673	SAMN12670393
	Mischonyx cuspidatus	MCZ-139265	This article	100,365,298	CGATGT	504	24,154	PRJNA556673	SAMN12670408
Sodreaninae	*Sodreana leprevosti*	MCZ-139256	This article	29,302,986	GATCAG	446	22,319	PRJNA556673	SAMN12670424
	Sodreana sodrena	MCZ-139268	This article	50,087,486	CAGATC	368	16,660	PRJNA556673	SAMN12670425
Progonyleptoidellinae	*Gonyleptoides marumbiensis*	MCZ-139254	This article	45,926,997	TGACCA	434	17,541	PRJNA556673	SAMN12670401
	Iporangaia pustulosa	MCZ-139275	This article	51,357,517	CTTGTA	431	20,837	PRJNA556673	SAMN12670405
	Progonyleptoidellus striatus	MZSP-71021	This article	28,188,757	TTAGGC	386	17,252	PRJNA556673	SAMN12670414
	Neosadocus maximus	MCZ-139257	This article	17,104,651	ACAGTG	441	17,688	PRJNA556673	SAMN12670410
Outgroups									
Sandokanidae	*Gnomulus rostratus*	MCZ-141286	Fernández et al.	10,146,702		412	20,861		SRR5242747
Phalangodidae	*Scotolemon lespesi*	MCZ-43672	This article	69,786,947	GATCAG	441	26,206	PRJNA556673	SAMN12670423
Assamiidae	*Dampetrus* sp.	MCZ-44061	Fernández et al.	12,850,673		763	5440		SRR5242745
Samoidae	*Pellobunus* sp.	MCZ-153028	Fernández et al.	23,303,809			13,591		SRR5242746
Kimulidae	*Tegipiolus pachypus*	MZSP-71018	This article	25,503,557	ATCACG	426	14,527	PRJNA556673	SAMN12670426
Biantidae	*Metabiantes* sp.	MCZ-49748	Fernández et al.	72,266,554			128		SRR1146667

the python script *selectslice.py* (Cunha and Giribet 2019). Matrix 1 (50% occupancy matrix) is represented by 2597 genes and Matrix 2 (85% occupancy matrix) contains 328 genes. To diminish the effects of saturation we used TrimAl v1.2 (Capella-Gutiérrez et al. 2009) in order to remove from Matrix 1 the 30% fastest evolving genes, resulting in a matrix with 1819 genes (Matrix 3). Phyutility (Smith and Dunn 2008) was used to concatenate the matrices.

Phylogenetic Analyses

For phylogeny estimation, we compared maximum likelihood (ML), Bayesian inference (BI), and coalescent-based (CB) estimation methods to account for possible differences between gene-tree/species-tree inferences. For analyses under ML, we used IQ-TREE-MPI v1.5.5 (Nguyen et al. 2015). We carried out a gene-partitioned analysis (PART) with model search including the LG4 mixture model and accounting for heterotachy for all matrices. In addition, we conducted a nonpartitioned analysis (CAT) with model search including the 10–60 profile mixture models (an ML variant of the Bayesian CAT model) for Matrix 2. Model search was carried out in Model Finder, incorporated in IQ-TREE (Kalyaanamoorthy et al. 2017).

For Matrix 2, we also carried out BI analyses in ExaBayes v. 1.21 (Aberer et al. 2014) with openmpi v. 1.64, and PhyloBayes MPI v1.7a (Lartillot et al. 2013). For ExaBayes, we ran four independent Markov Chain Monte Carlo (MCMC) for 500,000 generations, sampling every 500 generations and discarding 10% of the trees as burn-in for each MCMC run prior to convergence (i.e., when maximum discrepancies across chains < 0.1). For PhyloBayes we first recoded Matrix 2 into the six Dayhoff categories (Dayhoff et al. 1978) in order to speed up computation times and ameliorate possible artifacts related to long-branch attraction and taxon-specific compositional heterogeneity (Foster 2004; Cunha and Giribet 2019). We ran four independent chains with the CAT-GTR model (eliminating constant sites to speed up computation) for 308,836–335,923 cycles discarding as "burn-in" the first 30,000 generations. Convergence was assessed using the *bpcomp* and *tracecomp* programs in PhyloBayes. We considered that convergence was reached when *tracecomp* statistics dropped below 0.1 for all relative difference scores (rel_diff), effective samples sizes were > 300 (effsize), and *bpcomp* maximum discrepancy in clade support was < 0.1 (maxdiff). Detailed statistics for the PhyloBayes runs can be found in the Supplementary material S2 available on Dryad. We did not conduct analyses under BI for Matrices 1 and 3 due to their large size (2597 and 1819 orthogroups, respectively). For analyses under the coalescent-based (CB) approach, individual trees were generated for Matrices 1 and 2 with IQ-TREE1.6.beta4; following this we used ASTRAL-II v4.10.12. (Mirarab and Warnow 2015) to infer the species tree from all individual unrooted gene trees under the multispecies coalescent model.

Nodal support for analyses in IQ-TREE was calculated via 1000 ultrafast bootstrap replicates (-bb 1000; Hoang et al. 2018). For the ASTRAL analyses, we calculated local posterior probabilities which are computed based on gene tree quartet frequencies (Sayyari and Mirarab 2016).

Divergence Time Inference

The root of the tree was constrained using the 95% highest posterior density (HPD) of the chronogram for the 78-gene data set of Fernández et al. (2017), ranging between 253 and 117 Ma. The divergence between Gonyleptoidea/Assamioidea/Biantoidea/Zalmoxoidea and its sister group, Sandokanidae, was constrained by the only described Burmese amber laniatorean fossil, *Petrobunoides sharmai*. (Selden et al. 2016), a member of the SE Asian clade Epedanoidea, with a phylogenetic position as sister group of Gonyleptoidea/Assamioidea/Biantoidea/Zalmoxoidea (Fernández et al. 2017) or as the sister group of Assamiidae (Kury et al. 2019). We therefore used the date of the fossil (99 Ma) as the lower limit for our dating analyses and chose as upper limit the oldest age of the 95% HPD obtained by Fernández et al. (2017) for the age of the clade containing Assamioidea/Biantoidea/Zalmoxoidea/Gonyleptoidea, at 196 Ma. Two samoids, *Pellobunus insularis* and *Hummelinckiolus silhavyi* from Dominican amber (Cokendolpher 1986; Cokendolpher and Poinar 1998) were used to constrain *Pellobunus* with its sister group. The age of the Dominican amber is controversial (Iturralde-Vinent and MacPhee 1996) but it has been constrained to 16 Ma (Iturralde-Vinent 2001; Penney 2010), which we use as a lower limit, the upper limit being again the maximum age estimated by the 95% HPD in Fernández et al. (2017) for the divergence between Samoidae and its sister clade (Biantidae/Escadabiidae/Fissiphalliidae/Zalmoxidae), at 102 Ma.

We performed a node-calibrated Bayesian relaxed-clock dating in the program MCMCtree (Yang and Rannala 2006), part of the PAML 4.9 package (Yang 2007). First, we estimated in CODEML a rough substitution rate as well as a gradient (g) and Hessian (H) of the branch lengths without the clock (usedata=3). Then, we used the approximate likelihood method (usedata=2) to estimate divergence times (dos Reis and Yang 2011; dos Reis et al. 2017). We implemented the independent rate model, a birth–death prior on the divergence time, and the LG protein model with gamma rates among sites (Le and Gascuel 2008). C10 to C60 profile mixture models implicitly include a Gamma rate heterogeneity among sites (IQ-TREE manual).

We tested the effect of five different prior distributions for the node calibrations (uniform, skew-normal, skew-t, truncated-Cauchy, and gamma), to investigate the sensitivity of the results to the type of distribution. For each test, we used the same prior distribution on the three calibrated nodes and soft lower and upper bounds for the three calibration points. We decided

TABLE 2. Parameters implemented for the prior distributions for the node calibrations for each of the five analyses.

	Node 1	Node 2	Node 3
Skew-N	SN(1.22,0.58,50)	SN(1.04,0.41,50)	SN(0.21,0.36,50)
Uniform	>1.17<2.53 (specified in control file)	B(0.99,1.96)	B(0.16,1.02)
Cauchy	L(1.17,0.5,0.065,1e-300)	L(0.99,0.5,0.036,1e-300)	L(0.16,0.5,0.028,1e-300)
Skew-T	ST(1.17,0.053,50,1)	ST(0.99,0.038,50,1)	ST(0.16,0.034,50,1)
Gamma	G(3416.3,2690)	G(2932.1,2690)	G(699.4,2690)

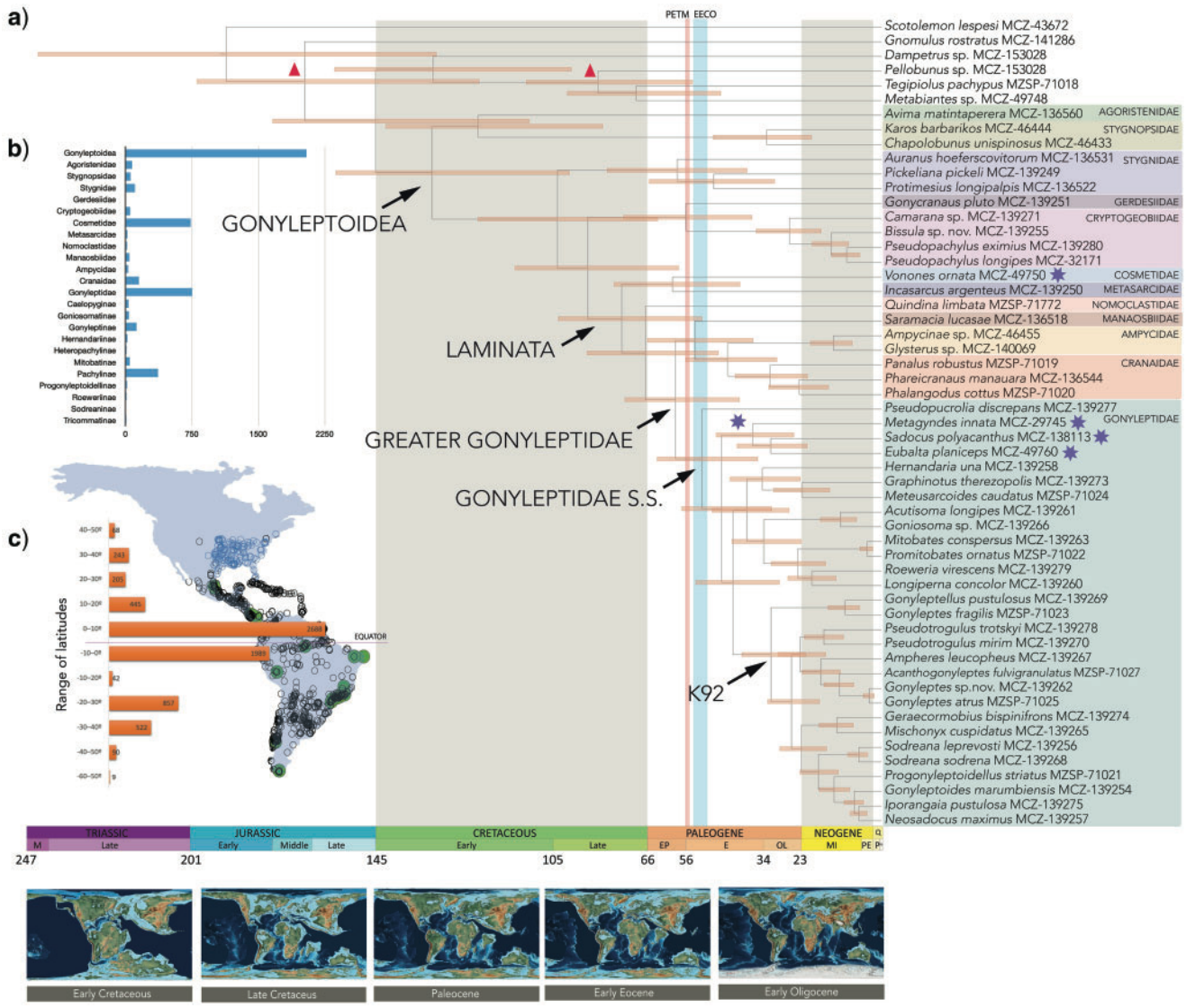


FIGURE 2. a) Chronogram of gonyleptoid evolution inferred from the analysis of Matrix 2 (210 orthogroups, 85% occupancy) in MCMCTREE with 95% highest posterior density (HPD) bars under a Uniform prior. Nodes calibrated with fossils are indicated with a red triangle. Purple stars as in Figure 1. b) Species level diversity for Gonyleptoidea and Gonyleptidae extracted from the World Catalogue of Opiliones (Kury et al. 2020). c) Latitudinal gradient starting from North to South in 10° interval for all the records of Gonyleptoidea available in GBIF. See Methods for details. Figure also shows Paleomap reconstructions (Scotese 2016) from the Early Cretaceous to the Early Oligocene. For reference, major global events are highlighted on the geological timeline (PETM, Paleocene–Eocene Thermal Maximum [pink bar]; EECO, Early Eocene Climatic Optimum [blue bar]) (Online version in color).

on this approach because we could not decide *a priori* which type of prior distribution was objectively better given our knowledge of the Opiliones fossil record.

We used the R package MCMCtreeR (Puttick 2019) to generate the time priors for MCMCtree analyses. Table 2 shows the parameters implemented for the different analyses; plots with the estimated age distributions

for the different parameters for the three calibrated nodes can be found in Supplementary material S3 available on Dryad. For each of our five analyses, two MCMC were run for 10 million generations, sampling every 50 generations after a burn-in phase of 1 million generations, using the topology given by the analysis of the 328 og matrix with IQ-TREE – CAT as a reference tree. As measures of diagnosing convergence of the MCMC chains we plotted the posterior means of run 1 versus run 2 for each analysis to confirm that both runs had converged on the same distribution (Supplementary material S4 available on Dryad) and used Tracer v1.7.1 (Rambaut et al. 2018) to ensured that all runs had effective sample sizes (ESS's) >200 (although some ESS values were >140). We ran an MCMC with no data for each analysis and generated prior and posterior density plots of node ages (Supplementary material S5 available on Dryad) to verify that the posterior densities were contained within the prior densities for the calibrated nodes (dos Reis and Yang 2019).

Analysis of Latitudinal Diversity

In order to test the existence of a latitudinal diversity gradient in the superfamily, one would need a properly curated database for the distribution of all the species in Gonyleptoidea, as typically done for other organisms, but this is difficult to extract from existing data. As a conservative approach, and because a database with species distributions does not exist (as is the case of spiders; Piel 2018), we downloaded all existing gonyleptoid GBIF records as of April 6th, 2020 (https://doi.org/10.15468/dl.63ylx9). The 19,532 references were binned in 10° latitudinal groups (Fig. 2c). This data set has caveats, especially as the occurrences could not be filtered to 1 per species, as many species were identified to genus or family, and because it over-represents North American samples, which for example, has many occurrences for the two species of US cosmetids (see Supplementary material S6 available on Dryad for a map of Gonyleptoidea distribution based on the records found in GBIF). It also has biases in the sampling efforts in some specific region, like the Brazilian Atlantic rainforest. Because the biases (over-representation of temperate samples and underrepresentation of tropical biodiverse regions) would not favor the existence of the LDG, the database could provide a conservative test to the LDG.

Results and Discussion

Matrix Composition

We used occupancy thresholds and evolutionary rates as criteria to select or exclude orthogroups in our matrices. For matrices 1 and 2, we used an occupancy threshold as a way to reduce orthogroups with too few representatives. For example, in the case of the 50% occupancy matrix (Matrix 1), an OMA orthogroup (og, sets of orthologous genes) is selected if it is present in at least 50% of the taxa (this is, 28 taxa). *Matrix 1* with 50% occupancy consisted of 2597 og and 806,809 aa (Fig. 1a). *Matrix 2* with 85% occupancy consisted of 328 og and 78,541 aa (Fig. 1a). For *Matrix 3*, we eliminated from Matrix 1 the 30% fastest evolving genes; it consisted of 1819 og and 586,667 aa.

Phylogenetics and Time of Divergence

Figure 1b shows the phylogeny inferred by the analysis of Matrix 2 with a nonpartitioned analysis under ML with and LG + C60 model as selected by ModelFinder. Remaining trees are available in the Supplementary material available on Dryad. All our matrices and analyses (ML, BI, and CB) support the monophyly of Gonyleptoidea, with the families Agoristenidae and Stygnopsidae branching early in the tree and a second lineage including the remaining gonyleptoid families (Fig. 1b). The remaining gonyleptoids diverged from their sister group during the Cretaceous (Fig. 2; Supplementary Fig. S7 available on Dryad; only the 95% HPD of the Uniform prior extends into the Late Jurassic), and include the members of (*a*) Stygnidae, (*b*) the clade Gerdesiidae + Cryptogeobiidae, sister group to (*c*) the lineage Laminata (Kury and Villarreal 2015), comprising Cosmetidae, Metasarcidae, Nomoclastidae, Manaosbiidae, Cranaidae, Ampycinae, and Gonyleptidae. Laminata has maximum support under all analyses and has been almost universally found in earlier molecular phylogenetic analyses (Giribet et al. 2010; Sharma and Giribet 2011; Pinto-da-Rocha et al. 2014; Bragagnolo et al. 2015; Wong et al. 2017). The relationship of Gerdesiidae + Cryptogeobiidae + Laminata has been also found in multilocus Sanger-based (Bragagnolo et al. 2015) and morphological studies (Kury and Villarreal 2015); according to the latter, Laminata was erected due to the position of Nomoclastidae as sister group to Microsetata, originally defined to include Cosmetidae, Metasarcidae, Manaosbiidae, Cranaidae, and Gonyleptidae based on the presence of microsetae on the surface of the ventral plate of the penis (Kury 2014). Our analyses are not compatible with the Microsetata hypothesis. Structure within Laminata—a lineage characterized by the presence of a *lamina ventralis* (or ventral setigerous plate) on the penis (Kury and Villarreal 2015) and that diversified most likely between the Late Cretaceous and the Eocene (Fig. 2; Supplementary Fig. S7 available on Dryad)—is likewise well supported and includes the clade Cosmetidae + Metasarcidae (also found with maximum support under all analytical conditions), sister group to Nomoclastidae plus a lineage called Greater Gonyleptidae or GG (Kury 2014) that comprises Gonyleptidae s.s. plus three taxa (Ampycinae, Cranaidae, and Manaosbiidae) forming a clade of Central American and Northern South American gonyleptoids. Our results thus differ from earlier work nesting Cranaidae and/or Ampycinae within Gonyleptidae s.s. (Pinto-da-Rocha et al. 2014;

Kury and Villarreal 2015) and justifies the use of Ampycidae at the familial level (Ampycidae *new rank*). Ampycinae was proposed as a new subfamily by Kury (2003) as it is well defined morphologically (Kury 2003; Kury and Alonso-Zarazaga 2011), but its phylogenetic position requires this new rank. We thus refer to these four clades as families: Gonyleptidae, Cranaidae, Manaosbiidae, and Ampycidae, equivalent to the unranked Greater Gonyleptidae of Kury.

Phylogenetics and Biogeography of Gonyleptidae

The phylogeny of gonyleptid species, excluding Ampycidae, shows taxonomic and biogeographic structure, and includes a relatively recent radiation of Neotropical species that initiated rapid diversification during the Paleocene/Eocene. The earliest splits within Gonyleptidae are between the northern Brazilian Heteropachylinae and the remaining species, and then by a clade of Chilean Pachylinae versus the rest, this Chilean clade of interest due to its adaptation to the temperate zone.

Structure within Gonyleptidae supports large clades proposed earlier based on ecological, behavioral, and chemical characters, such as the K92 clade of Caetano and Machado (2013), comprised of Caelopyginae, Gonyleptinae, Hernandariinae, Progonyleptoidellinae, and Sodreaninae, although, as in the case of Pinto-da-Rocha et al. (2014), neither Gonyleptinae nor Progonyleptoidellinae are monophyletic. Progonyleptoidellinae is paraphyletic with respect to the Gonyleptinae *Neosadocus*, with the same structure presented in Pinto-da-Rocha et al. (2014). This position of *Neosadocus* is intriguing since its external morphology resembles more that of other "Gonyleptinae" rather than Progonylleptoidellinae, but given ours and previous analyses (Pinto-da-Rocha et al. 2014), we propose the transfer *Neosadocus* to Progonyleptoidellinae (*new subfamilial assignment*). Also, as in the latter study, certain Gonyleptinae (including *Acanthogonyleptes* and some *Gonyleptes*) appear related to Caelopyginae and Hernandariinae, while others (including another *Gonyleptes* and *Gonyleptellus*) are the sister group to all the remaining members of the K92 clade. This brings no surprise, as Gonyleptinae is poorly diagnosed morphologically, lacking any clear synapomorphy. Our representatives of the CM12 clade (Caetano and Machado 2013) (Mitobatine + Goniosomatinae) are sister group to a clade that includes a species of Hernandariinae (*Hernandaria una*) and two species of Pachylinae, failing to support Hernandariinae as a clade, as *H. una* does not group with *Pseudotrogulus*.

Pachylinae comprises almost 400 species in more than 120 genera, but no morphological synapomorphies have been proposed for this subfamily. We included here only five genera, which do not necessarily reflect the morphological disparity of the group. Even with such poor taxon coverage, Pachylinae is not monophyletic, as it was the case in previous phylogenetic analyses (morphological or molecular) (Hara et al. 2012; Pinto-da-Rocha et al. 2014). The inclusion of more groups from different South American regions is urgently needed to better understand the relationships among members of this large group.

Out of the Tropics, or How to Become a Temperate Species

Our analyses show a clade including the temperate Pachylinae, restricted to Chile and the Argentinean border, to which we refer to as Pachylinae s.s. This clade was recovered in previous studies comprising seven genera (including species of the type-genus *Pachylus* and our *Sadocus* and *Metagyndes*) (Pinto-da-Rocha et al. 2014; Bragagnolo et al. 2015) and evidences colonization of the now temperate zone early in the history of the group. Indeed, the temperate Pachylinae diverged from their sister group in the Paleogene (between the Paleocene and the Eocene), and the represented species started diversifying between Eocene and the Oligocene (Fig. 2; Supplementary Fig. S7 available on Dryad), at a time when the Neotropical area extended southward into regions now considered temperate (Romero 1986; Bernardes-de-Oliveira et al. 2014). Indeed, the Paleocene–Eocene Thermal Maximum (PETM), ~56 Mya, was a period of carbon release characterized by an increase in temperature (by 5–8°C) and shifts in faunal and floral composition worldwide (Sluijs et al. 2007; McInerney and Wing 2011), which promoted diversification in some clades of terrestrial arthropods (Lackner et al. 2019). The PETM precedes the temperate Pachyline and slightly overlaps with the 95% HPD for three of our calibration priors for the origin of the temperate Pachylinae, thus, although not providing an explanation, could suggest a possible mechanism for the OTT hypothesis, or how tropical groups may become temperate (Jablonski et al. 2006) overcoming physiological limitations to climate. Indeed, the Central Chilean region was tropical to subtropical during the Paleogene (Barreda et al. 2007). As forests extended from today's southern Brazil into Argentina and Chile, a tropical clade of Pachylinae got established in this region and later became isolated by the uplift of the Andes in the late Miocene–Pliocene (Barreda et al. 2007), the region becoming temperate as the planet cooled down. This scenario for the colonization of the extratropics is both consistent with the tree topology of Gonyleptidae and also explains how the latitudinal diversity gradient can be established by origination in the tropics and dispersal to the extratropics over evolutionary timescales (Krug et al. 2009) A complete phylogeny of Gonyleptoidea may be however necessary to further test this hypothesis, as it has been done in other groups of organisms (Condamine et al. 2012; Rolland et al. 2014).

Conclusions

In this study, we used phylotranscriptomics and divergence time dating to infer the evolutionary history of the

most diverse superfamily of Opiliones, the Neotropical Gonyleptoidea. This phylogenetic and temporal framework allowed us to better understand the expansion of a tropical clade of Gonyleptoidea into the extratropics and provided evidence for a single net colonization of the temperate region over a large timescale in the southern hemisphere, perhaps suggesting that persistence in the extratropics may be difficult for organisms that evolved in the tropics.

Supplementary Material

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.d2547d815.

Matrices and tree files are available in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S27034). New raw reads are deposited in the NCBI Sequence Read Archive (Bioproject PRJNA556673, Biosamples SAMN12670385–SAMN12670426, SAMN 14113753).

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