Phylogenomics of darkling beetles (Coleoptera: Tenebrionidae) from the Atacama Desert (#77131)

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Phylogenomics of darkling beetles (Coleoptera: Tenebrionidae) from the Atacama Desert

Lapo Ragionieri [Corresp., Equal first author, 1], **Alvaro Zúñiga-Reinoso** [Equal first author, 1], **Marcel Blaeser** [1], **Reinhard Predel** [Corresp. 1]

[1] University of Cologne Institute of Zoology, Cologne, Germany

Corresponding Authors: Lapo Ragionieri, Reinhard Predel
Email address: lapo.ragionieri@uni-koeln.de, rpredel@uni-koeln.de

Background. Tenebrionidae (Insecta: Coleoptera) are a conspicuous component of desert fauna worldwide. In these ecosystems, they are significantly responsible for nutrient cycling and show remarkable morphological and physiological adaptations. Nevertheless, Tenebrionidae colonizing individual deserts have repeatedly emerged from different lineages. The goal of our study was to gain insights into the phylogenetic relationships of the tenebrionid genera from the Atacama Desert and how these taxa are related to the globally distributed Tenebrionidae.

Methods. We used newly generated transcriptome data (47 tribes, 7 of 11 subfamilies) that allowed for a comprehensive phylogenomic analysis of the tenebrionid fauna of this hyperarid desert and fills a gap in our knowledge of the highly diversified Tenebrionidae. We examined two independent data sets known to be suitable for phylogenomic reconstructions. One is based on 34 neuropeptide precursors, the other on 1742 orthologous genes shared among Coleoptera.

Results. The majority of Atacama genera are placed into three groups, two of which belong to typical South American lineages within the Pimeliinae. While the data support the monophyly of the Physogasterini, Nycteliini and Scotobiini, this does not hold for the Atacama genera of Edrotini, Epitragini, Evaniosomini, Praociini, Stenosini, Thinobatini, and Trilobocarini. A suggested very close relationship of *Psammetichus* with the Mediterranean *Leptoderis* could also not be confirmed. We also provide hints regarding the phylogenetic relationships of the Caenocrypticini, which occur both in South America and southern Africa. Apart from the focus on the Tenebrionidae from the Atacama Desert, we found a striking synapomorphy grouping Alleculinae, Blaptinae, Diaperinae, Stenochinae, and several taxa of Tenebrioninae, but not *Tenebrio* and *Tribolium*. This character, an insertion in the *myosuppressin* gene, defines a higher-level monophyletic group within the Tenebrionidae.

Conclusion. Transcriptome data allow a comprehensive phylogenomic analysis of the tenebrionid fauna of the Atacama Desert, which represents one of the seven major endemic tribal areas in the world for Tenebrionidae. Most Atacama genera could be placed in three lineages typical of South America; monophyly is not supported for several tribes based on molecular data, suggesting that a detailed systematic revision of several groups appears necessary.

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Lapo Ragionieri[1*], Álvaro Zúñiga-Reinoso[1*], Marcel Bläser[1], Reinhard Predel[1]

[1] Institute for Zoology, University of Cologne, Cologne, Germany
[*]authors contributed equally

Corresponding Authors:
Lapo Ragionieri
Álvaro Zúñiga-Reinoso
Reinhard Predel

Zülpicher Str. 47b, 50674 Cologne, Germany
Email address: lapo.ragionieri@uni-koeln.de; azunigar@uni-koeln.de; rpredel@uni-koeln.de.

Abstract

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Introduction

The beetle family Tenebrionidae Latreille, 1802 (Insecta: Coleoptera) has a worldwide distribution and is one of the larger families with more than 30,000 described species (Bouchard *et al*., 2021). In the majority of species, both larvae and adults are detritivores and often play a significant role in terrestrial food webs (Matthews *et al.*, 2010). Based on their ecological preferences the Tenebrionidae can be broadly divided into two groups: species associated with trees and species with a shift in larval habitat from decaying trees to soil (Matthews *et al.,* 2010). The latter are widely recognized as the insect group best suited for colonizing arid environments and are found worldwide in desert ecosystems. They have developed numerous morphological, physiological and behavioural adaptations to cope with extremely arid conditions and are therefore largely responsible for most of the nutrient cycling in deserts (Cloudsley-Thompson & Chadwick, 1964; Cloudsley-Thompson, 2001; Crawford, 1982; Matthews, 2000; Matthews *et al.*, 2010; Cheli *et al*., 2022). Different from most other insect groups, their biodiversity sometimes increases with increased aridity (Kergoat *et al.*, 2014a; Koch, 1962; Pfeiffer & Bayannasan, 2012). The genetic basis for these desert adaptations is not yet clear, but it is known that different lineages of the Tenebrionidae have repeatedly migrated into developing deserts in a convergent scenario (Matthews *et al.*, 2010). Currently, 11 subfamilies, 106 tribes and 2307 genera of Tenebrionidae are recognized (Bouchard *et al.*, 2021), mainly based on the morphological characters of adults and larvae (Doyen, 1972, 1993; Doyen & Tschinkel, 1982; Kamiński *et al.,* 2020; Matthews *et al.*, 2010; Watt, 1974).

Recent analyses in insect phylogeny resolved the higher-level relationships in many cases using extensive molecular datasets (*e.g.,* Chesters, 2020; Misof et *al.,* 2014; Wipfler *et al.,* 2019). The intra-ordinal relationships in Coleoptera (Bocak *et al.*, 2014; Cai *et al*., 2022; Gunter *et al.*, 2014; Hunt *et al.*, 2007; McKenna *et al.*, 2019; Zhang *et al.*, 2018) and the intra-familial relationships of the larger beetle families (*e.g.,* Tarasov & Dimitrov, 2016; Nie *et al.*, 2020; Shin *et al.*, 2018; Souza *et al.*, 2020) was also the focus of several such studies. Regarding the Tenebrionidae, unresolved relationships were repeatedly addressed by molecular analyses in recent years, which, among others, consistently confirmed the monophyly of the family (Gunter *et al.*, 2014; Kergoat *et al.*, 2014b; Kaminski *et al*., 2020). However, these phylogenetic reconstructions are still under discussion because the internal relationships are still not fully solved. In particular, the subfamilies Tenebrioninae Latreille, 1802 and Diaperinae Latreille, 1802 appear to be artificial groups that require thorough revaluation. (*e.g.,* Aalbu *et al.,* 2002; Kergoat *et al.,* 2014b; Kamiński *et al.,* 2020; Johnston *et al*., 2020). A recent study convincingly suggested the

subfamily Blaptinae Leach, 1815 as a monophyletic group based on molecular and morphological analyses (Kamiński *et al.,* 2020); this lineage contains taxa that have traditionally been placed within the presumably polyphyletic subfamily Tenebrioninae. One of the limitations of all phylogenetic reconstructions is the lack of comprehensive sampling of lineages from Africa and southern South America. Both Africa and South America each have a highly conspicuous tenebrionid fauna including several endemic tribes (*e.g.,* Carrara & Flores, 2015; Koch, 1962; Kuschel, 1969; Matthews *et al.*, 2010) and contain two of the oldest and driest deserts in the world, the Namib and Atacama Deserts (Clarke, 2006; Goudie & Eckardt, 1999) where tenebionids represent one of the most conspicuous insect group.

Aridity in the Atacama Desert can be traced to the Triassic, but the current conditions are closely related to the Andes uplift in the Miocene (Clarke, 2006), because this mountain range acts as an effective rain shadow (Houston & Hartley, 2003). The regions west of the Andes experienced a long-term decrease in precipitation in this context; the corresponding aridification presumably started in the early Miocene in what is now the core area of the Atacama Desert (Dunai et al. 2005; Ritter *et al.,* 2018) and intensified throughout the Miocene until the present (Jordan et al. 2014, Ritter *et al.,* 2018). Today, the core of the Atacama Desert (Central Depression between 19°S-23°S) is characterized by hyperarid conditions with less than 2 mm/yr of precipitations (Houston, 2006), making it one of the driest regions on Earth (Clarke, 2006). These climatic conditions are apparently a barrier for the evolution of organisms, and even well-adapted xerophilous insects as darkling beetles avoid the core of the Atacama Desert. Indeed, most tenebrionids prefer peripherally located and slightly wetter habitats in the Coastal and Andean Cordilleras (Fig. 1). However, the long-lasting interactions between tectonic activity and past climate changes in Atacama Desert created conditions for the diversification of a very peculiar fauna of tenebrionids, some with very ancient relationships (see Endrödy-Younga, 1996 and Ferrer, 2015); and under the influence of the fauna of neighboring regions of the Peruvian Desert and the Intermediate Desert of Coquimbo (Peña, 1966).

The main goals of the current study are obtaining insights 1) into the diversification of tenebrionids in the Atacama, 2) into the phylogenetic relationships of the Atacama genera, and 3) of the relationships of these taxa to Tenebrionidae from other regions. For this purpose, we collected material for molecular analyses of almost all tenebrionid genera (30 genera including an undescribed genus of Alleculinae Laporte, 1840) that inhabit the Chilean Atacama Desert including the adjacent Andean Cordillera. Since it is unlikely that analyses of individual genes can resolve all issues concerning the higher phylogeny of the Tenebrionidae, we sequenced transcriptomes of tenebrionid genera from the Chilean Atacama Desert throughout. In addition to the transcriptomes of the Tenebrionidae from the Atacama Desert, the transcriptomes of a larger number of tenebrionid genera from other regions of the world were sequenced to improve taxon sampling for our transcriptome analyses. Finally, our dataset includes seven of the 11 described subfamilies and 47 tribes. We used these data to obtain the deduced amino acid sequences from 34 neuropeptide precursors per species. The suitability of neuropeptide precursor sequences for phylogenetic inferences was previously demonstrated in a proof-of-concept study (Bläser *et al.*,

2020). This approach is relatively fast and simple as it is based on a limited set of easily identifiable and well conserved protein coding genes. In an alternate analysis using the same transcriptome dataset, the rather commonly used approach of compiling a large scale dataset of 1742 orthologous genes was performed. Both approaches, the concatenated dataset of neuropeptide precursors and the large scale dataset of orthologous genes were thus used in parallel to evaluate the relationships within the Atacama Tenebrionidae. These analyses resulted in maximum support for most, but not all branches and enabled a first convincing assessment of the origin and phylogenetic relationships of the Tenebrionidae of the Atacama Desert.

Materials & Methods

2.1 Insect collection

Tenebrionid beetles from the Chilean Atacama Desert (30 genera, 14 tribes) were collected by hand between 2017 and 2021 (Table 1; collecting permits CONAF N°005/2017, 105/2020, 016/2021). The collected specimens were either transferred directly into 96% ethanol for DNA and RNA analyses or transported alive for RNA extraction from fresh material; RNA extraction was then carried out in the Cologne laboratory. Furthermore, we collected samples of 51 tenebrionid genera (33 additional tribes) from Central Chile (collecting permits CONAF N°005/2017), Germany, Italy, Spain, Portugal (collecting permit N° 757-758/2021/CAPT), Namibia (collecting permit NCRST RPIV01042034) and Peru (collecting permits SERFOR Nr D000019-2022) to improve taxon sampling for phylogenetic analyses. In addition, published peptide precursor sequences of *Tribolium castaneum* (Herbst, 1797) (Triboliini Gistel, 1848), *Zophobas atratus* (Fabricius, 1775) (Tenebrionini Latreille, 1802) (Marciniak *et al.,* 2022) and *Tenebrio molitor* Linnaeus, 1758 (Tenebrionini) (Li *et al.*, 2008; Veenstra, 2019; Marciniak *et al.,* 2022) were added to our dataset, while peptide precursor sequences of *Neomida bicornis* (Fabricius, 1777) (Diaperinae: Diaperini Latreille, 1802) were obtained by Blast searches in the NCBI database (https://www.ncbi.nlm.nih.gov/Traces/wgs?val=GDMA01). RNA was additionally extracted from seven taxa of Tenebrionoidea Latreille, 1802 (families Ciidae Leach, 1819, Meloidae Gyllenhaal, 1810, Mycetophagidae Leach, 1815, Pyrochroidae Latreille, 1807, Salpingidae Leach, 1815, Zopheridae Solier, 1834) and one Cleroidea (Melyridae Leach, 1815) (Table 1), which were included in the phylogenetic analyses. Taxonomic determination was carried out by Álvaro Zúñiga-Reinoso and Reinhard Predel.

2.2 RNA extraction, cDNA library preparation and sequencing

Total RNA was extracted from samples stored in absolute ethanol or from individuals kept alive until tissue dissection. To avoid excessive RNA degradation in specimens stored in ethanol, head and pronotum of the beetles were separated from the rest of the body before transferring them into ethanol. In larger species, the body was additionally opened longitudinally with sterilized scissors. Without any treatment prior to storage in ethanol, the RNA was usually highly degraded, suggesting limited penetration of ethanol across the cuticle. Grinding of whole insects was avoided in order to enable the intestine to be removed later. Insects alive until tissue

dissection were kept at 4 °C for 10 minutes before preparation. In most individuals (both ethanol and fresh material), after removal of the appendages (legs, elytra, antennae), the body was opened dorsally with sterilized scissors, the intestine was removed and the central nervous system (CNS) was carefully dissected. In small species, representing the genera *Ammobius* Guérin-Méneville, 1844, *Achanius* Erichson, 1847*, Colydium* Fabricius, 1792*, Cordibates* Kulzer, 1956*, Corticeus* Piller & Mitterpacher, 1783*, Dichillus* Jacquelin du Val, 1861*, Discopleurus* Lacordaire, 1859*, Eledona* Latreille, 1796*, Melanimon* Steven, 1829*, Oochrotus* Lucas, 1852*, Synchita* Hellwig, 1792*,* and *Thinobatis* Eschscholtz, 1831, the CNS was not dissected. For all other samples, total RNA was extracted from CNS and remaining tissues separately using 1 mL of TRIzol (Thermo Fisher Scientific, Darmstadt, Germany) following the manufacturers recommendations. Total RNA from each sample was quantified using Qubit RNA Assay Kit (Thermo Fisher Scientific) and subsequently subjected to quality control and RNA integrity number (RIN) as implemented in the Agilent 2100 Bioanalyzer system (Agilent Technologies, Waldbronn, Germany). Finally, RNA from CNS and remaining tissue from each sample were pooled together in equimolar concentrations for library preparations. This approach improved the detection of peptide precursor sequences, whose genes are mainly expressed in the CNS. Sequencing libraries (double-indexed) were prepared using 1 µg of total RNA with the Illumina® TruSeq® stranded RNA sample preparation kit (Cat.20020594; Illumina, San Diego, CA, U.S.A.). If the total RNA concentration was insufficient for standard library preparation, at least 2 ng of extract was pre-amplified using the Ovation RNA-Seq System V2 (NuGen, San Carlos, CA, USA). The library preparation of pre-amplified samples was performed according to the Nextera XT DNA sample preparation protocol (part no. 15031942 Rev. C). Subsequent sample preparation and sequencing was carried out at the Cologne Center for Genomics on an Illumina HiSeq 4000 and Illumina NovaSeq 6000 systems as described in Ragionieri & Predel (2020) with 75 bp or 100 bp paired end reads.

2.3 Transcriptome assembly, evaluation of cross-contaminations and statistics

Raw data (FASTQ files format) were filtered by removing adapter sequences and low quality using Trimmomatic 0.38 (Bolger *et al.*, 2014). The resulting filtered RAW reads were submitted to NCBI (Sequence Read Archives (SRA): pending; BioProject: pending). Filtered reads were *de novo* assembled using Trinity v2.2.0 (Grabherr *et al.*, 2011; Haas *et al.*, 2013) with the read normalization option. All transcriptome assemblies were checked for potential cross-contaminations due to multiplex sequencing of several libraries using CroCo v1.1 (Simion *et al.*, 2018) which removes potential sources of contamination using both transcriptome assemblies and the corresponding paired raw data (Table S1). This strategy uses sequence similarities and abundances to detect potential cross-contaminations. For closely related species that are analysed together, this can lead to an overestimation of cross-contamination (Simion *et al*., 2018). CroCo was run with the following settings: fold-threshold 2, minimum-coverage 0.1, overexp FLOAT 300, minimum percent identity between two transcripts to suspect across contamination 98%, minimum length of an alignment between two transcripts to suspect a cross contamination 180. Finally, we checked for and eliminated additional contamination of vector and linker/adapter

using UniVec database (http://www.ncbi.nlm.nih.gov/tools/vecscreen/univec/). The transcriptomes assembled in this study lost on average about 2% of their sequence information due to the cross-contamination check. The quality and the completeness according to conserved single-copy ortholog content of transcriptome assemblies were evaluated using the Perl script (TrinityStats.pl) included in Trinity and BUSCO v3 based on an Endopterygota obd9 dataset (Seppey *et al.*, 2019), respectively. The filtered transcriptome assemblies were submitted to NCBI Transcriptome Shotgun Assembly database (Table 1) and used for the large scale data set phylogenetic reconstruction.

2.4 Orthology assessment and alignment of neuropeptide precursors

Available amino acid sequences of neuropeptide precursors of *Tr. castaneum* and *Te. molitor* (Li *et al.*, 2008; Veenstra, 2019; Marciniak *et al.,* 2022) were used as initial queries to search for orthologous sequences in the transcriptome assemblies. The assembled transcripts were analysed with the tblastn algorithms provided by NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) or BioEdit version 7.0.5.3 (Hall, 1999). In case of missing data, precursor sequences of closely related taxa were used as alternative query sequences. Candidate nucleotide precursor gene sequences were translated into amino acid sequences using the ExPASy Translate tool (Artimo *et al.*, 2012; http://web.expasy.org/translate/) with the standard genetic code. Orthologous neuropeptide precursor sequences were aligned using the MAFFT-L-INS-i algorithm (Katoh and Standley, 2013) (dvtditr (amino acid) Version 7.299b alg=A, model=BLOSUM62, 1.53, -0.00, -0.00, noshift, amax=0.0); terminal sequences which were only found in few species were manually trimmed. The results were then manually checked for misaligned sequences using, *e.g.,* N-termini of signal peptides and conserved amino-acid residues (cleavage signals, Cys as target for disulfide bridges) as anchor points. Individual amino acid alignments of each group of orthologous neuropeptide precursors were concatenated with catsequences 1.3 (https://zenodo.org/record/4409153#.YmJYT35Byot). The average evolutionary divergence for each neuropeptide precursor was calculated as in Bläser & Predel (2020). Briefly, overall mean distances (± standard error after 500 bootstrap generations) were computed with MEGA X (Kumar *et al.*, 2018) implementing the Poisson correction model (Zuckerkandl & Pauling, 1965). Amino acid compositions and parsimony informative sites of the combined alignment were calculated using MEGA X.

2.5 Compilation of an orthologous gene dataset of Tenebrionidae

A Coleoptera orthologous reference gene set was compiled using OrthoDB v10. This approach provides reliable markers for phylogenomics (Misof *et al.,* 2014; MecKenna *et al.,* 2019). Single copy genes shared across species of Coleoptera (Taxonomy ID: 7041) were selected for analysis. Orthograph (Petersen *et al.,* 2017) was used to generate a profile hidden Markov model from the amino acid sequences of transcripts of each reference gene on the filtered transcriptome assemblies. Initially, we obtained 2689 orthogroups (OGs) shared among Coleoptera, which were subsequently aligned using the MAFFT-L-INS-I algorithm (Katoh & Standley, 2013). Alignment ambiguities or spurious sequences in each OG were identified and removed using

trimAL 1.2 (Capella-Gutierrez et al., 2009) with residue overlap threshold (-resoverlap 0.75) and sequence overlap threshold (-seqoverlap 90). With that approach, 947 out of 2689 OGs were removed from the initial data set. Finally, all OGs were concatenated in a single partitioned super-alignment using catsequences.

2.6 Genome sequencing, assembly and identification of *myosuppressin* genes

Whole genome extraction was carried out using thoracic muscles of a single individual of *Nycterinus abdominalis* Eschscholtz, 1829 collected in Talcahuano, Chile. High molecular weight genomic DNA was purified using MagAttract® HMW DNA Kit (Ref. 67563, QIAGEN GmbH, Hilden, Germany). DNA concentration was determined using Qubit 2.0 Fluorometer (Thermo Fisher Scientific). Fragment size was verified using DNA integrity number as implemented in the Agilent 2100 Bioanalyzer system. Genomic DNA library was prepared using the Illumina TruSeq Nano DNA High Throughput Library Prep Kit (Illumina, Cat. No 20015965) with modifications of the protocol (TruSeq DNA Nano Reference Guide, Document # 1000000040135 v00, October 2017). Only one cycle of polymerase chain reaction (PCR) was conducted to complete adapter structures in order to avoid PCR bias. Library validation and quantification were carried out as implemented in Agilent TapeStation, and subsequently the library was pooled and quantified using the Peqlab KAPA Library Quantification Kit (Roche Sequencing Solutions, Inc., USA; KK4835-07960204001) on an Applied Biosystems 7900HT Sequence Detection System and finally sequenced on an Illumina NovaSeq 6000 sequencer with 150 bp paired end reads. Raw data (FASTQ files format) were filtered by removing adapter sequences and low quality reads using Trimmomatic 0.38 (Bolger *et al.*, 2014). Filtered raw data were assembled using the programs SOAPdenovo2 (Luo *et al.,* 2012) using different k-mer values. The myosuppressin precursor was identified as described above (2.4). Genomic nucleotide sequences containing introns were subsequently aligned manually in BioEdit version 7.0.5.3 (Hall, 1999).

2.7 Phylogenetic analysis of neuropeptide precursors and a large scale orthologous gene dataset

FASTA files of aligned peptide precursor sequences were converted into PHYLIP and NEXUS formats using AliView 1.18-beta7 (Larsson et al., 2014). After defining the N-terminus of each neuropeptide precursor as starting partition, best-fit partitioning schemes and substitution models for subsequent phylogenetic analyses were predicted with ModelFinder (Chernomor *et al.,* 2016; Kalyaanamoorthy *et al.*, 2017; Minh *et al.*, 2021) implemented in IQ-TREE release 2.1.4b (Minh *et al.*, 2020). Models and concatenated alignments for all analyses of both data sets are listed in Data S1 and S2. All phylogenetic analyses have been rooted using the Cleroidea *Melyris* sp.. Bayesian inference (BI) analyses were run with MrBayes, with four runs, using eight chains and a sample frequency of 1,000 until convergence was achieved (PSFR value between 1.00 – 1.02) with a 10,000,000 generations (Ronquist *et al*., 2012). Maximum likelihood (ML) analyses were carried out using IQ-TREE 2.1.4b. ML analyses of both data sets were ran with the nearest-neighbour interchange search to consider all possible nearest-neighbour interchanges (-allnni)

and branch support was evaluated with 1,000 ultra-fast bootstrap (UFBoot) (Hoang et al., 2018) and the Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT) (Guindon et al., 2010). Trees were visualized using FigTree 1.4.2 (http://tree.bio.ed.ac.uk/) and designed in Inkscape 1.0 (https://inkscape.org/).

Results

About 34 native genera of Tenebrionidae were described from the Chilean Atacama Desert (Ferrú & Elgueta, 2011; Peña, 1966a; Vidal & Guerrero, 2007); the exact number depends on the definition of the boundaries of the Atacama (see Fig. 1). We collected and analysed specimens of 30 genera (*Epipedonota* Solier, 1836*, Conibius* LeConte, 1851 and *Parepitragus* Casey, 1907 missing), including genera that inhabit only peripheral regions such as the high Andes (*Antofagapraocis* Flores, 2000*, Pilobalia* Burmeister, 1875 and an undescribed genus of Alleculinae) or the salty beaches and dunes of the Pacific coast (*Phaleria* Latreille, 1802, *Thinobatis*). In addition, sequence data of introduced species were obtained either from publicly available databases (*Te. molitor*, *Tr. castaneum*) or the beetles were sequenced from breeding strains (*Alphitobius diaperinus* (Panzer, 1797)). The analysed taxa from the Atacama Desert are currently classified in five subfamilies (Alleculinae, Blaptinae, Diaperinae, Pimeliinae Latreille, 1802, Tenebrioninae) and 17 tribes (Table 1). For an assessment of the phylogenetic position of the Atacama genera, we additionally generated a transcriptome dataset encompassing diverse tenebrionid taxa (altogether seven of the 11 described subfamilies, 47 tribes) from other regions of the world (Table 1), taxa of different families belonging to the superfamily Tenebrionoidea (Ciidae, Meloidae, Mycetophagidae, Pyrochroidae, Salpingidae, Zopheridae), and Melyridae.

A) Analysis of neuropeptide precursors. The primary matrix comprises 6457 amino acids from 34 neuropeptide and neuropeptide-like precursors; information on sequence length and sequence coverage is provided in Table S2. The average evolutionary divergence over all sequences of the precursor dataset is 0.25 (± 0.03) and differs considerably between the different precursors (Table S2). The best fitting models according to ModelFinder are listed for each partition in Data S1, which also contains the concatenated alignment.

The phylogenetic tree of the concatenated neuropeptide precursor dataset (Fig. 2) recovered Tenebrionidae as monophyletic. Tenebrionidae are separated into one clade containing all the Pimeliinae analysed and a second clade containing the taxa of Alleculinae, Blaptinae, Diaperinae, Lagriinae Latreille, 1825, Stenochinae Kirby, 1837, and Tenebrioninae. All Atacama genera of Pimeliinae belong to a clade which is recovered as sister to *Akis* Herbst, 1799 (Akidini Billberg, 1820) and *Pimelia* Fabricius, 1775 (Pimeliini Latreille, 1802) from the Mediterranean region. The Atacama Pimeliinae are separated into a lineage containing Chilean genera of Elenophorini Solier, 1837, Nycteliini Solier, 1834, Physogasterini Lacordaire, 1859, Praociini Eschscholtz, 1829, and Stenosini Schaum, 1859 and a second lineage including all remaining Pimeliinae from the Atacama Desert. Internal branches of the first clade generally show high support. This clade is first divided into a group containing Stenosini and a group containing the

Elenophorini, Nycteliini, Physogasterini, and Praociini from the Atacama Desert. Within the Stenosini with the Chilean *Discopleurus* and *Hexagonochilus* Solier, 1851 nests Mediterranean *Leptoderis* Billberg, 1820 (Elenophorini) as sister to Mediterranean *Dichillus* (Stenosini). The only described member of the Elenophorini from the Atacama region, *Psammetichus* Latreille, 1829, appears on a branch with the southern African *Eurychora* Thunberg, 1789 (Adelostomini Solier, 1834) and Mediterranean *Sepidium* Fabricius, 1775 (Sepidiini Eschscholz, 1829). The remaining taxa of this large clade branch into Mediterranean *Glabrasida* Escalera, 1910 (Asidini Fleming, 1821) and the Nycteliini, Physogasterini, and Praociini from the Atacama. The Nycteliini, which appear as sister to Praociini and Physogasterini, are represented by the genera *Auladera* Solier, 1836, *Callyntra* Solier, 1836*, Nyctelia* Laterille, 1825, and *Psectrascelis* Solier, 1836*,* and the sister taxa thereof, *Gyriosomus* Guérin-Méneville, 1834+ *Pilobalia*. While the Physogasterini *Philorea* Erichson, 1834 + (*Physogaster* Lacordaire, 1830 + *Entomochilus* Solier, 1844) occur as monophyletic in our analysis, the Praociini are polyphyletic, with *Gyrasida* Koch, 1962 as sister to (*Praocis* Eschscholtz, 1829 + *Falsopraocis* Kulzer, 1958) + Physogasterini. *Antofagapraocis* occurs as sister to the latter group. The topology of the second lineage with Pimeliinae from the Atacama Desert shows *Caenocrypticoides* Kaszab, 1969 (Caenocrypticini, Koch 1958) separated from the rest. The remaining taxa split into a heterogeneous group comprising southern African Zophosini Solier 1834 and Adesmiini Lacordaire, 1859, Mediterranean Erodiini Billberg, 1820, and Tentyriini Eschscholtz 1831 and a branch with the Atacama genera of Edrotini Lacordaire 1859, Epitragini Blanchard, 1845, Evaniosomini Lacordaire, 1859, Thinobatini Lacordaire 1859, and Trilobocarini Lacordaire, 1859. Within the latter branch the topology shows with maximum branch support the evaniosomin *Melaphorus* Guérin-Ménéville, 1834 + *Evaniosomus* Guérin-Ménéville, 1834 and *Aryenis* Bates, 1868 as sister to *Trilobocara* Solier, 1851 (Trilobocarini) and these four taxa appear as sister to the rest of this clade. Within these remaining taxa the epitragin *Geoborus* Blanchard, 1842 + *Nyctopetus* Guérin-Ménéville, 1831 (Central Chile) and *Salax* Guérin-Méneville, 1834 (Trilobocarini) are sister to *Achanius*, *Arthroconus* Solier, 1851, *Aspidolobus* Redtenbacher, 1868, *Cordibates*, *Eremoecus* Lacordaire, 1859, *Hylithus* Guérin-Méneville, 1834, and *Thinobatis*. While [*Hylithus* (Edrotini) + *Thinobatis* (Thinobatini)] + *Cordibates* (Thinobatini) form a well-supported monophyletic group, the sister group relationships of *Achanius* (Evaniosomini), *Arthroconus* (Edrotini), *Aspidolobus* (Epitragini), and *Eremoecus* (Trilobocarini) are not fully resolved. While both phylogenetic inferences support a topology with *Eremoecus* + *Aspidolobus* as sister to *Hylithus* + [*Thinobatis* + *Cordibates*], *Achanius* + *Arthroconus* are sister to the above mentioned group (Fig. 2, Fig. S1) but the branch supports are rather low.

In the sister group of the Pimeliinae, the three analyzed taxa of Lagriinae (incl. Adeliini Kirby, 1828, Cossyphini Latreille, 1802, Lagriini Latreille 1825; without representatives in the Atacama Desert) form the sister group to the remaining species of this clade, which in turn is separated into *Tenebrio* + [*Bolitophagus* Illiger, 1798 + *Eledona* Latreille, 1797] from Europe (both Bolitophagini Kirby 1837) and the rest. The latter group contains *Tribolium* + European *Melanimon* (Melanimini Seidlitz, 1894) as sister to the remaining taxa. These remaining taxa are

further divided into Blaptinae (incl. Blaptini Leach, 1815, Opatrini Brullé, 1832), Pedinini Eschscholtz, 1829, Platynotini Mulsant & Rey, 1853) with *Blapstinus* Dejean, 1821 (Opatrini) from the Atacama Desert and a second clade which consists of Alleculinae, Diaperinae, Stenochinae, and several Tenebrioninae. The first branch of that diverse clade separates European *Nalassus* Mulsant, 1854 (Tenebrioninae: Helopini Latreille 1802) from the rest, which is further separated into Stenochinae (without representatives in the Atacama Desert) and a clade consisting of Alleculinae, Diaperinae, and Tenebrioninae. Members of Diaperinae (Crypticini Brullé, 1832 and Hypophlaeini Billberg, 1820 from Europe) form together with *A. diaperinus* (Tenebrioninae) the sister to the rest. The latter clade splits into monophyletic Alleculinae (incl. Alleculini Laporte, 1840, Cteniopodini Solier, 1835) with an undescribed species from the periphery of the Atacama Desert (Alleculinae gen. nov.) and a subclade containing further Diaperinae and Tenebrioninae. The Diaperinae of this subclade, including *Phaleria* (Phaleriini Blanchard, 1845) from the beaches of the Atacama Desert and Holarctic Diaperini, are sister to the Mediterranean *Scaurus* Fabricius, 1775 and a clade consisting of Scotobiini Solier 1838 /Amphidorini LeConte, 1862 from the Atacama Desert and the Neotropical *Z. atratus* (Tenebrionini). Within the latter group the genus *Nycterinus* Eschscholtz, 1829 (*incertae sedis*) is sister to Scotobiini (*Ammophorus* Guérin-Méneville, 1830 + [*Scotobius* Germar, 1824 + *Diastoleus* Solier, 1838]) and *Z. atratus*.

Overall, in the neuropeptide tree few branches show low support (Fig. S1). These branches include the position of *Achanius* to *Arthroconus* (SH-aLRT = 3.4, UFBoot = 43), *Salax* as sister to *Nyctopetus* + *Geoborus* (SH-aLRT = 14.7, UFBoot = 65), *Praocis* + *Falsopraocis* (SH-aLRT = 51.4, UFBoot = 89), *Auladera* as sister to *Callyntra* + *Psectrascel*is (SH-aLRT = 38, UFBoot = 79), *Sepidium* + *Psammetichus* (SH-aLRT = 3, UFBoot = 47), *Diaperis* Geoffroy, 1762 + *Neomida* Latreille, 1829 (SH-aLRT = 6.9, UFBoot = 86), *Nestorinus* Guerrero, Vidal & Zúñiga-Reinoso, 2022 + *Heliofugus* Guérin-Méneville 1831 (SH-aLRT = 28.9, UFBoot = 82), *Isomira* Mulsant, 1856 as sister to *Omophlus* Dejean, 1834 + *Heliotaurus* Mulsant, 1856, and Diaperini/Phaleriini as sister to a clade with Scotobiini/*Prionychus* Solier, 1835 /*Zophobas* Dejean, 1834 + Scaurini Billberg, 1820 (SH-aLRT = 62.9, UFBoot = 91).

All analysed taxa of Alleculinae, Blaptinae, Diaperinae, and Stenochinae, as well as those taxa of Tenebrioninae that nest within the sister clade of Blaptinae, have a distinct synapomorphy in common, namely an insertion of eight amino acids in the myosuppressin precursor (Fig. 3A; see Data S3 for full sequences). This insertion does not result from differential transcription, but it is indeed manifested at the gene level. This could be verified by genome sequencing of a *N. abdominalis* specimen and a subsequent comparison of the *myosuppressin* gene structures (exons) of *N. abdominalis* and *Tr. castaneum* (Fig. 3B).

B) Analysis of a large scale dataset of orthologous genes. The partitioned and concatenated alignment is composed of 1742 OGs with an overall length of 788676 amino acid sites (Data S2). The best fitting models according to ModelFinder are listed for each partition in Data S2, which also contains the concatenated alignment. The topology of the resulting tree (Fig. 4) is

largely congruent with that of the neuropeptide precursor data set. Differences are mainly observed for several of those branches with low support in the neuropeptide precursor tree (see Fig. S1): *Salax* as sister to a clade comprising *Achanius*, *Arthroconus*, *Aspidolobus*, *Cordibates*, *Eremoecus*, *Hylithus,* and *Thinobatis*; *Praocis* as sister to *Falsopraocis* + Physogasterini; *Auladera* + *Nyctelia* as sister to *Callyntra* + *Psectrascelis*; *Sepidium* as sister to *Psammetichus* + *Eurychora*; Alleculinae as sister to Scotobiini/*Nycterinus*/*Zophobas* + Scaurini; *Nestorinus* as sister to *Heliofugus* + *Cuphotes* Champion, 1887; and *Isomira* + *Prionychus* as sister to *Omophlus* + *Heliotaurus*. In addition, *Discopleurus* is sister to a main branch of Pimeliinae (Fig. 4), including, among other tribes, also the Stenosini; and *Nycterinus* changed its position and was recovered as sister to *Diastoleus* + *Scotobius*. In the large scale data set of orthologous genes, the branches with low support (Fig. S2) include that with *Zophobas as* sister to *Nycterinus*, *Scotobius* and *Diastoleus* (SH-aLRT = 8.2/ UFBoot = 61). In both data sets, *Corticeus* has the same position, but the corresponding branches are very long.

Discussion

In South America, two major biogeographical regions are recognized, the Neotropical and the Andean regions, which are separated by the South American Transition Zone (Morrone, 2014). The Atacama Desert is located in the Transition Zone, where the fauna and flora of both regions partially overlap (Morrone, 2015). There are several possible scenarios for the origin of the current tenebrionid fauna of the Chilean Atacama. (1) The beetles are recently introduced species. From our focus area cosmopolitan genera such as *Alphitobius*, *Gnathocerus* Thunberg, 1814, *Palorus* Mulsant, 1854, *Tenebrio,* and *Tribolium* were recorded (Ferru & Elgueta, 2011), mostly from the Arica region with its extensive riverbed plantations. Due to their close association with human settlements, these genera are not treated here as native or invasive taxa. Three of these genera (*Alphitobius*, *Tenebrio*, *Tribolium*) are included in our transcriptome datasets, but the corresponding specimens were not collected in Chile. (2) The emergence of the Isthmus of Panama, led to the migration of North American tenebrionids into the already hyperarid Atacama Desert. The exact date is still not conclusively resolved, but most assumptions suggest that the connection between the continents of North and South America has existed for about three million years (O´Dea et al. 2016) and caused the Great American Biotic Interchange (Cody *et al.*, 2010; Wilson *et al.*, 2014; Woodburne, 2010). However, several authors suggest an even older connection between the Americas, with an initial land bridge existing about 23 Ma (Bacon et al. 2015) or between 15 and 6 Ma (Bacon et al. 2015, Montes et al.2015). Finally, (3) the fauna of the Atacama Desert can be traced back to long separated originally Gondwanan elements, which then developed independently for at least 120 million years. Several paleoendemic relicts of Gondwanan origin are known for South America and in particular Chile, which were probably already adapted to arid conditions before the breakup of Gondwana. Among them are insects of the tribe Cicindini Bänninger, 1927 (Coleoptera: Carabidae; Kavanaugh and Erwin, 1991), *Heterolepisma* Escherich, 1905 and *Stylifera* Stach, 1932 (Zygentoma: Lepismatidae; Mendes, 2018), *Maindronia* Bouvier, 1897 (Zygentoma: Maindroniidae; Wygodzinsky, 1940; Zúñiga-Reinoso & Predel, 2019), spiders of the genus

Cyrioctea Simon, 1889 (Grismado & Pizarro-Araya, 2016) and plants of the family Zygophyllaceae R. Br. (Shmida, 1985).

Phylogenetic relationships of the Atacama genera of Tenebrionidae

Transcriptomic information, mostly obtained from single individuals, was on the one hand used to obtain the amino acid sequences of 34 orthologous peptide precursors of genera of Tenebrionidae from the Atacama Desert and of selected taxa from other regions of the world. Due to their co-evolution with their corresponding receptors, neuropeptide sequences are particularly conserved and very well suited for a reconstruction of phylogenetic relationships at the intra-ordinal level (Bläser *et al.*, 2020; Predel *et al.*, 2012; Roth *et al.*, 2009). Other advantages of using such datasets are the ease of ortholog assignment and the presence of unambiguous and highly conserved sequence motifs that facilitate a manual control of alignments generated by sequence alignment programs. The parallel analysis of the large scale dataset of orthologous genes revealed mostly the same topology as the neuropeptide precursor tree, with the exception of the few differences discussed below. The majority of Atacama genera cluster in three clades. Two of these clades belong to the subfamily Pimeliinae, which contains most of the desert-adapted darkling beetles worldwide (Doyen, 1993; Kergoat *et al.*, 2014b). In the Pimeliinae *Pimelia*/*Akis* were found to be the sister group to the remaining 17 analyzed tribes of Pimeliinae. The latter lineage consists of two clades, each containing a larger number of Atacama genera. One of these clusters with a larger number of Atacama taxa contains Nycteliini, Praociini and Physogasterini and forms a well-supported monophyletic group. This confirms previous morphological studies, which suggested Praociini, Physogasterini and Nycteliini as closely related taxa (Doyen, 1972, 1993). These tribes are only known from arid regions of South America and are thought to be the sister group of North American Coniontini Waterhouse, 1858, Branchini LeConte, 1862 and Asidini (Doyen, 1993). The Mediterranean *Glabrasida* representing Asidini, was recovered in our analyses as sister to Praociini, Physogasterini and Nycteliini. Different from the most recent cladistic analysis of morphological characters in Nycteliini (Flores, 2000a), our analysis shows monophyletic Nycteliini as sister to Praociini + Phyogasterini. Within Nycteliini, which generally avoid the hyperarid core of the Atacama Desert, *Pilobalia* + *Gyriosomus* form the sister clade to the remaining Nycteliini. The latter three genera do not occur in the Atacama Desert or adjacent regions, but are otherwise widely distributed in southern South America. Physogasterini represent another very well supported monophyletic group in our analyses and include many species typical of the hyperarid core of the Atacama Desert. However, Praociini as defined in Flores (2000b) and Flores & Vidal (2009) appear polyphyletic with both data sets, this tribe requires a re-evaluation based on molecular data. From the four genera included here, *Praocis* and *Falsopraocis* are sister to Physogasterini, while *Antofagapraocis* and the central Chilean *Gyrasida* do not form a monophyletic group with *Praocis* and *Falsopraocis*. The genus *Psammetichus*, which is typical of hyperarid environments along the coastal Cordillera of the Atacama Desert and the Pampa de Tamarugal, belongs to a sister clade of the above tribes. That clade also includes Sepidiini and Adelostomini, which do

not occur in South America (Bouchard et al. 2021). Kamiński et al. (2022) suggested Sepidiini and Adelostomini as closely related tribes, considering the morphology of female terminalia and several genes. However, they did not place Elenophorini close to these tribes. *Psammetichus* was transferred to Elenophorini by Doyen and Lawrence (1979), a tribe that also includes *Leptoderis* (= *Elenophorus* Dejean 1821) of the western Mediterranean. *Leptoderis* was also included in our transcriptomic dataset, but the molecular data do not support an ancient link. In fact, *Psammetichus* was kept in Elenophorini in the past, although it was never found to be closely related to *Leptoderis* in Doyen's cladograms (Doyen, 1993). Also Ferrer (2015) doubts this relationship due to a number of morphological characters not shared between *Leptoderis* and the South American Elenophorini. In our tree, *Leptoderis* robustly nests within Stenosini, the latter represented by Chilean *Discopleurus* (within Stenosini only in the neuropeptide tree) and *Hexagonochilus*, and the palaearctic *Dichillus* as sister to *Leptoderis*.

The second major branch of Pimeliinae excl. *Akis/Pimelia* has *Caenocrypticoides* as sister to the rest. *Caenocrypticoides* is a well-established example of members of the same tribe (here Caenocrypticini; Endrody-Younga, 1996) occurring in widely separated arid regions of Africa and South America, and thus probably representing a relict pattern that points to xerophilic ancestors before the break-up of Gondwana. The sister clade of *Caenocrypticoides* diverges into one lineage with diverse taxa having a wide distribution in the Palaeartic and Africa, but are not present in South America (Erodiini, Tentyriini, Zophosini, Adesmiini) and a second lineage with South American taxa belonging to Edrotini, Epitragini, Evaniosomini, Thinobatini, and Trilobocarini. The current placement of genera within these tribes is based on morphological characters (*e.g.,* Doyen, 1993; Flores & Aballay, 2015). Although the exact position, particularly those of *Arthroconus* (Edrotini), *Salax* (Trilobocarini) and *Achanius* (Evaniosomini) could not be fully resolved with our data, it is obvious that none of the tribes is monophyletic. This South American clade was already mentioned by Doyen (1993) as a group "not easy to fit with any classification" using morphology and the classification at tribe level of the different genera have seen several changes over time (see e.g., Flores & Aballay, 2015). Doyen (1993) himself suggested transferring *Achanius* to the Edrotini (=Eurymetopini Casey, 1907). The first split in this lineage separates *Evaniosomus*/*Melaphorus*/*Aryenis* (Evaniosomini) + *Trilobocara* (Trilobocarini) from the remaining taxa with maximum branch support. These remaining taxa include, among others, *Achanius*, *Eremoecus*, and *Salax* (Trilobocarini) and thus further genera of the aforementioned tribes and are separated in the neuropeptide tree into *Geoborus*/*Nyctopetus* (Epitragini) + *Salax* and a subclade which, in addition to *Achanius*, *Arthroconus* and *Eremoecus*, also includes *Aspidolobus* as another representative of the Epitragini. In the large scale dataset of orthologous genes, *Salax* is sister to all above mentioned taxa, including *Geoborus* + *Nyctopetus*. Finally, the well supported sister group relationship of *Hylithus* (Edrotini) and *Thinobates* (Thinobatini) clearly argues against the supposed monophyly of Thinobatini which is only composed of the two genera included in our study (Doyen, 1993; Bouchard *et al.*, 2021).

The sister group of Pimeliinae contains all other tenebrionid taxa analyzed in our study. The basal branching separates Lagriinae from the rest, which shows an early branching of *Tenebrio* + Bolitophagini and *Tribolium* + Melanimini. The remaining taxa split into the recently re-established Blaptinae *sens. nov.* (Kamiński *et al*., 2020) incl. *Blapstinus* from the Atacama Desert, and a diverse group of taxa including Stenochinae, Diaperinae, Alleculinae, and Tenebrioninae. *Blapstinus* appears to be the only tenebrionid genus from the Atacama Desert that has close relatives in North America. The corresponding subtribe Blapstinina Mulsant & Rey, 1853 is in fact restricted to Nearctic and Neotropical regions (Lumen *et al*., 2020, Kaminski *et al*., 2022). Monophyly of the analyzed taxa of Lagriinae, Blaptinae, Stenochinae, and Alleculinae was confirmed with maximum branch supports, respectively. On the other hand, polyphyly was evident for Diaperinae and Tenebrioninae (see also, *e.g.,* Gunter *et al.*, 2014; Kergoat *et al.*, 2014b; Kamiński *et al*., 2020). Most taxa of the darkling beetles currently grouped in the subfamilies Alleculinae, Blaptinae, Diaperinae, Stenochinae, and Tenebrioninae have well-developed hindwings and do not show particular adaptations to hyperarid environments (Doyen, 1993). This does not apply to the Scotobiini, which represent the only endemic tribe of Tenebrioninae in arid South America (Matthews *et al.*, 2010) and include the third cluster of tenebrionid genera in the Atacama Desert. In fact, three of the six genera of Scotobiini (*Scotobius, Diastoleus, Ammophorus*) inhabit the Atacama Desert and were included in our analysis. Within this clade *Scotobius* + *Diastoleus* is sister to *Ammophorus* in the neuropeptide tree, whereas in the large scale data set of orthologous genes *Nycterinus* replaces the position of *Ammophorus.* While the classification within Scotobiini of *Diastoleus* and the widespread *Scotobius* has been stable, the systematic position of the genus *Ammophorus* changed considerably over time. When Solier (1838) established the Scotobiini, he included *Ammophorus* in this tribe. Shortly afterwards Lacordaire (1859) transferred this genus to Nyctoporini Lacordaire, 1859 (Pimeliinae), where it remained for over 100 years (see *e.g.,* Kulzer, 1955; Peck, 2006; Peña, 1966b). Later, Vidal & Guerrero (2007) transferred *Ammophorus* to Elenophorini (Pimeliinae). Based on detailed analyses of morphological characters, Doyen (1993) and Silvestro *et al.,* (2015) proposed to return the genus to Scotobiini. The result of the neuropeptide tree fits the placement of *Ammophorus* within Scotobiini based on morphology (Silvestro *et al*. 2015). Also, they share a peculiar synapomorphy with the presence of dome-shaped placoid sensilla on the last segment of the antennae (Doyen 1993). As sister of Scotobiini appears in the neuropeptide tree *Zophobas* Dejean, 1834 which is known only from Central and tropical South America (Ferrer, 2011). *Nycterinus* which is historically listed as the only South American genus within Amphidorini (see Doyen & Lawrence, 1979), belongs to the same monophyletic group in both data sets and was identified as sister to the above mentioned Scotobiini + *Zophobas* in the neuropeptide tree. Recent molecular phylogeny also showed *Nycterinus* as not belonging to the North American Amphidorini tribe, but rather to the South American Scotobiine clade which also includes Scotobiini and *Zophobas* (Johnston *et al*., 2022). The different results of the two data sets do not yet allow us to determine the specific position for *Nycterinus*.

The highly scattered appearance of the Tenebrioninae across the phylogenetic tree may question the reliability of our results. However, the topology does not show a mixture of taxa with poorly resolved sister group relationship, nor is it the result from particular poor taxon sampling. With the taxon-specific insertion of eight amino acids into the myosuppressin precursor (see Fig. 3) we have found a distinct synapomorphy at the molecular level clearly supporting Alleculinae, Blaptinae, Diaperinae, Stenochiinae, and a number of Tenebrioninae as a higher level monophyletic group. Based on morphological examinations, Doyen and Tschinkel speculated already in 1982 that Diaperinae, Stenochiinae, and Alleculinae could be derived offshoots of Tenebrioninae. Nevertheless, it does not seem an easy task to redefine any clade as Tenebrioninae except that which includes *Tenebrio* and Bolitopagini in our analyses.

Conclusions

Using newly generated transcriptome data, we were able to perform a comprehensive phylogenomic analysis of the tenebrionid fauna of the Atacama Desert and fill a gap in our knowledge of the highly diversified Tenebrionidae. The two datasets used for our analyses show few discrepancies that might be a more extensive taxon sampling. The majority of Atacama genera are placed into three groups, two of which belong to typical South American lineages within the Pimeliinae. The suggested very close relationship of *Psammetichus* with the Mediterranean *Leptoderis* was not confirmed. Caenocrypticini including the Chilean *Caenocrypticoides* comprises a small group of genera present in southern Africa and (mostly) the Andean region of South America. These taxa display a combination of characters shared with various clades (Doyen, 1993). Our results provide the first evidence for a position of *Caenocrypticoides* as the sister of one of the main branches within Pimeliinae. While our data support the monophyly of the Nycteliini, Physogasterini and Scotobiini, this does not hold for the Atacama genera of Edrotini, Epitragini, Evaniosomini, Praociini, Thinobatini, Stenosini, and Trilobocarini. To clarify the relationships of these taxa, it is certainly useful to include more southern South American representatives in future analyses. In general, a detailed systematic revision of each of the latter groups appears necessary. As a side effect of our study, we have found a striking synapomorphy grouping Alleculinae, Blaptinae, Diaperinae, Stenochinae, and several taxa of Tenebrioninae, but not *Tenebrio* and *Tribolium*. This character, an insertion in the *myosuppressin* gene, defines a higher-level monophyletic group within the Tenebrionidae.

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Legends Supplementary Material:

Supplemental Figure S1: Phylogenetic trees resulting from BI and ML analyses of the partitioned 34 neuropeptide and neuropeptide–like precursors from 83 genera of Tenebrionidae, including the 30 genera from the Atacama Desert. A) BI tree with posterior probability values for each branch. B) ML tree with bootstrap support values for each branch (SH-aLRT / UFBoot).

Supplemental Figure S2. ML tree of the partitioned amino acid supermatrix of 1742 OGs. Each node with branch support values SH-aLRT / UFBoot.

Supplemental Table S1. Cross-contamination and statistics of newly sequenced transcriptomes.

Supplemental Table S2. Neuropeptide precursors used in this study, including their completeness in the various taxa and the average evolutionary divergence across all sequence pairs in the 91 genera (including outgroup taxa).

Supplemental Data S1. Directory including:

- Matrix for ML analysis presented in Fig. 2 and Fig. S1 (amino acids in PHYLIP format).
- Matrix for BI analysis presented in Fig. 2 and Fig. S1, including partitions and evolutionary models for each partition from ModelFinder (amino acids in NEXUS format).
- Partition schemes of IQ-TREE matrix for Fig. 2 and Fig. S1.

Available at (DOI: 10.5880/CRC1211DB.35)

Supplemental Data S2. Directory including:

- Matrix for ML analysis presented in Fig. 4 and Fig S2 (amino acids in PHYLIP format).
- Partition schemes of IQ-TREE matrix for Fig. 4 and Fig. S2.

Available at (DOI: 10.5880/CRC1211DB.35)

Supplemental Data S3. Alignment with full sequences of the myosuppressin precursor motif shown in Fig. 3.

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Figure 1

Overview of the study area

Overview of the study area in the Atacama Desert (shaded area). This region and the adjacent Andean Cordillera are home to about 34 genera of Tenebrionidae, whose phylogenetic relationships are analysed in this study. Also shown are selected representatives of individual genera. Number of Atacama species and total number of species within the genera are noted, respectively. The dotted blue line is the 4,000 m.a.s.l. contour line in the west and the dashed red line is the average annual rainfall isohyet of 2 mm. The lower panel shows an elevation profile within the study area, exemplified for a cross-section south of Antofagasta (green line) with tenebrionids typical of different elevation levels along this transect.

Physogaster: 12/15

Geoborus: 1/2

Nycterinus: 5/20

Psammetichus: 10/14

Scotobius: 15/61

Gyriosomus: 6/44

Blapstinus: 1/>100

Antofagapraocis: 1/2

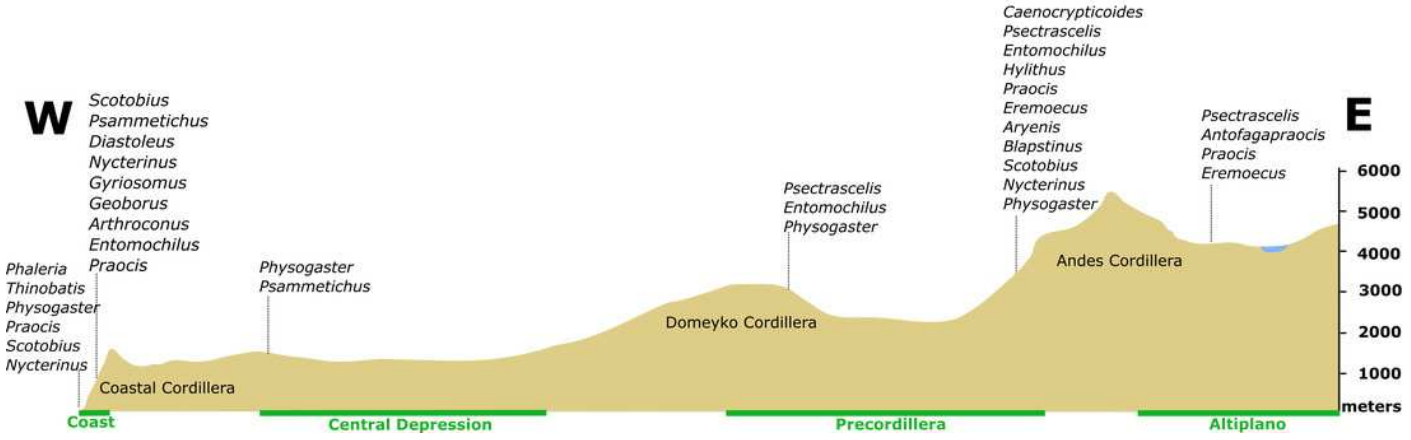

Figure 2

Neuropeptide trees

BI tree obtained from the analysis of a dataset of 34 peptide precursors from 83 genera of Tenebrionidae (47 tribes, seven subfamilies), including the 30 genera from the Atacama Desert. Assignment of subfamilies and tribes according to Matthews *et al.* (2010), Bouchard *et al.* (2021) and Kaminski *et al*. (2020); Color coding: Alleculinae, yellow; Blaptinae, pink; Diaperinae, light blue; Lagriinae, dark green; Pimeliinae, dark blue; Stenochinae, light green; Tenebrioninae, red. Atacama genera are marked with asterisks. Posterior probability (PP) and UFBoot (Bt) values are highlighted with circles on the nodes: black, above or equal to 0.95/95; grey, between 0.90-0.94/90-94; white, below 0.90/90. The detailed information on posterior probability / UFBoot values as well as the ML tree are provided in Fig. S1.

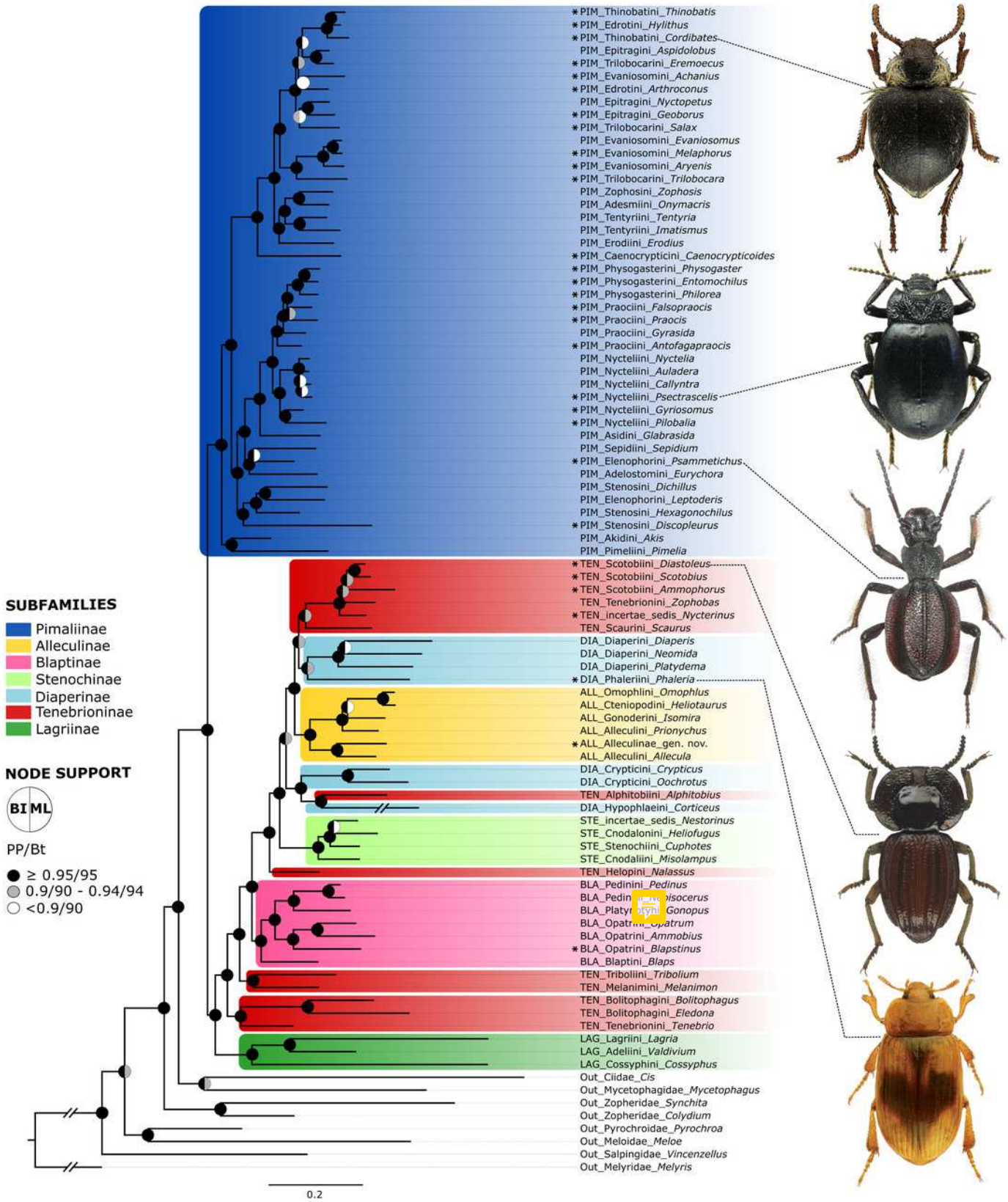
SUBFAMILIES
Pimaliinae
Alleculinae
Blaptinae
Stenochinae
Diaperinae
Tenebrioninae
Lagriinae
NODE SUPPORT
BI ML
PP/Bt
≥ 0.95/95
0.9/90 - 0.94/94
<0.9/90
PIM_Thinobatini_Thinobatis
PIM_Edrotini_Hylithus
PIM_Thinobatini_Cordibates
PIM_Epitragini_Aspidolobus
PIM_Trilobocarini_Eremoecus
PIM_Evaniosomini_Achanius
PIM_Edrotini_Arthroconus
PIM_Epitragini_Nyctopetus
PIM_Epitragini_Geoborus
PIM_Trilobocarini_Salax
PIM_Evaniosomini_Evaniosomus
PIM_Evaniosomini_Melaphorus
PIM_Evaniosomini_Aryenis
PIM_Trilobocarini_Trilobocara
PIM_Zophosini_Zophosis
PIM_Adesmiini_Onymacris
PIM_Tentyriini_Tentyria
PIM_Tentyriini_Imatismus
PIM_Erodiini_Erodius
PIM_Caenocrypticini_Caenocrypticoides
PIM_Physogasterini_Physogaster
PIM_Physogasterini_Entomochilus
PIM_Physogasterini_Philorea
PIM_Praociini_Falsopraocis
PIM_Praociini_Praocis
PIM_Praociini_Gyrasida
PIM_Praociini_Antofagapraocis
PIM_Nycteliini_Nyctelia
PIM_Nycteliini_Auladera
PIM_Nycteliini_Callyntra
PIM_Nycteliini_Psectrascelis
PIM_Nycteliini_Gyriosomus
PIM_Nycteliini_Pilobalia
PIM_Asidini_Glabrasida
PIM_Sepidiini_Sepidium
PIM_Elenophorini_Psammetichus
PIM_Adelostomini_Eurychora
PIM_Stenosini_Dichillus
PIM_Elenophorini_Leptoderis
PIM_Stenosini_Hexagonochilus
PIM_Stenosini_Discopleurus
PIM_Akidini_Akis
PIM_Pimeliini_Pimelia
TEN_Scotobiini_Diastoleus
TEN_Scotobiini_Scotobius
TEN_Scotobiini_Ammophorus
TEN_Tenebrionini_Zophobas
TEN_incertae_sedis_Nycterinus
TEN_Scaurini_Scaurus
DIA_Diaperini_Diaperis
DIA_Diaperini_Neomida
DIA_Diaperini_Platydema
DIA_Phaleriini_Phaleria
ALL_Omophlini_Omophlus
ALL_Cteniopodini_Heliotaurus
ALL_Gonoderini_Isomira
ALL_Alleculini_Prionychus
ALL_Alleculinae_gen. nov.
ALL_Alleculini_Allecula
DIA_Crypticini_Crypticus
DIA_Crypticini_Oochrotus
TEN_Alphitobiini_Alphitobius
DIA_Hypophlaeini_Corticeus
STE_incertae_sedis_Nestorinus
STE_Cnodalonini_Heliofugus
STE_Stenochiini_Cuphotes
STE_Cnodaliini_Misolampus
TEN_Helopini_Nalassus
BLA_Pedinini_Pedinus
BLA_Opatrini_Ammobius
BLA_Opatrini_Blapstinus
BLA_Blaptini_Blaps
TEN_Triboliini_Tribolium
TEN_Melanimini_Melanimon
TEN_Bolitophagini_Bolitophagus
TEN_Bolitophagini_Eledona
TEN_Tenebrionini_Tenebrio
LAG_Lagriini_Lagria
LAG_Adeliini_Valdivium
LAG_Cossyphini_Cossyphus
Out_Ciidae_Cis
Out_Mycetophagidae_Mycetophagus
Out_Zopheridae_Synchita
Out_Zopheridae_Colydium
Out_Pyrochroidae_Pyrochroa
Out_Meloidae_Meloe
Out_Salpingidae_Vincenzellus
Out_Melyridae_Melyris
0.2

Figure 3

Myosuppressin precursor sequences

Taxon-specific insertion in the myosuppressin precursor sequence, which represents a synapomorphy of a subgroup of Tenebrionidae. A) Simplified overview of a partial transcript sequence (see Data S3 for full sequences) showing the insertion in genera belonging to different subfamilies (Alleculinae, Blaptinae, Diaperinae, Stenochinae, Tenebrioninae). *N. abdominalis* position marked with * and *Tr. castaneum* position marked with +. B) Part of the corresponding gene sequence of the *myosuppressin* gene in *N. abdominalis* (analysed in this study) and the orthologous gene of *Tr. castaneum* (Li *et al*., 2008) without that sequence. Color coding: Alleculinae, yellow; Blaptinae, pink; Diaperinae, light blue; Lagriinae, dark green; Pimeliinae, dark blue; Stenochinae, light green; Tenebrioninae, red.

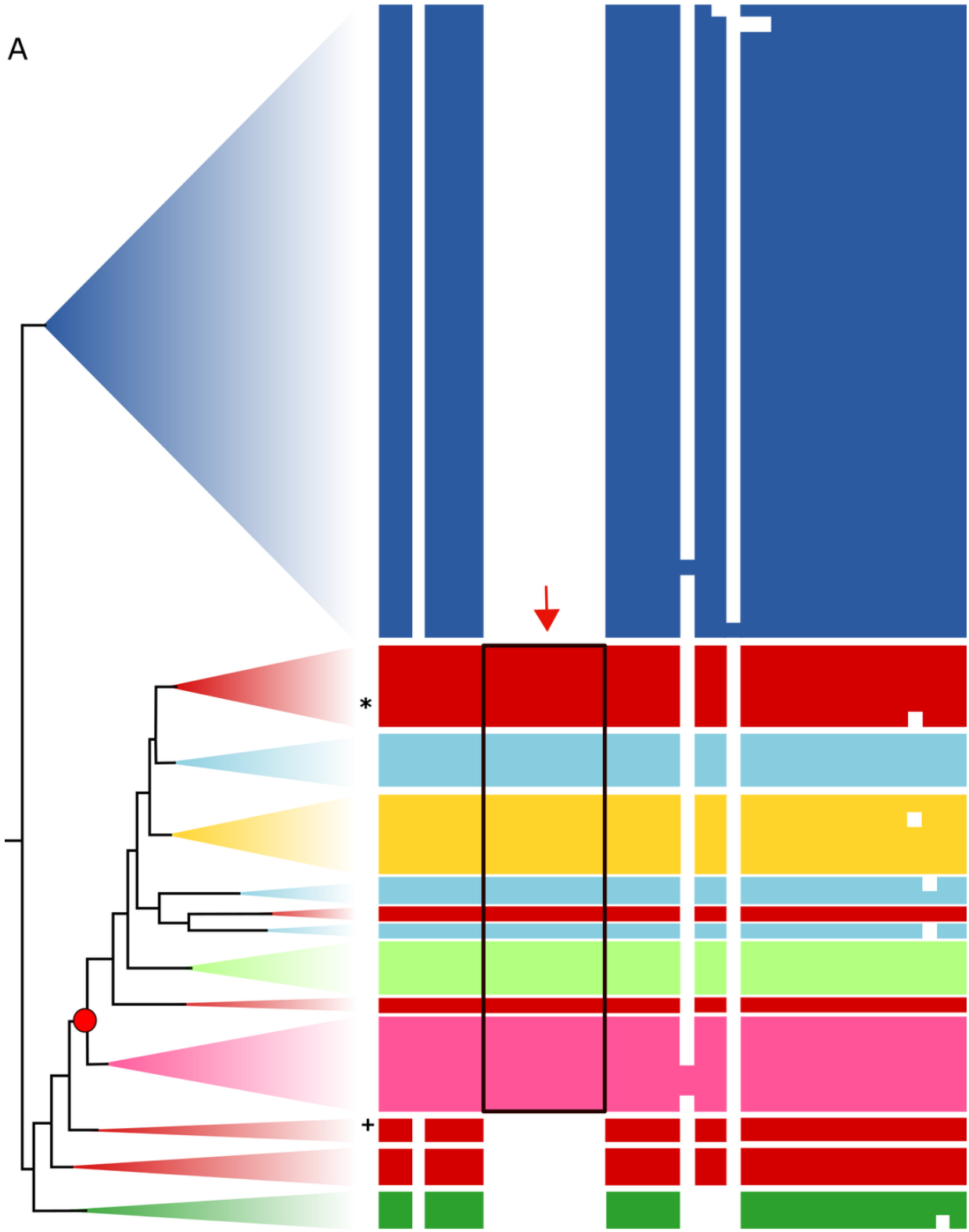
A
*
+
B
Nycterinus *
ttccagataggagagtggtggaacgcgatgtgagcccactggctgaaaggaacgtcaac
Amino acid
V E R D V S P L
Tribolium +
ttacagttcggagaatt----------gtgggaaggaatgtgaac

Figure 4

Orthogroups tree

ML phylogenetic tree obtained from the analysis of a dataset of 1742 orthogroups from 83 genera of Tenebrionidae, including the 30 genera from the Atacama Desert. Red squares mark species with different positions compared to the neuropeptide tree, the arrow shows the position of the Alleculinae clade as sister to Scotobiini + Scaurini. Color coding and branch support as in Figure 2.

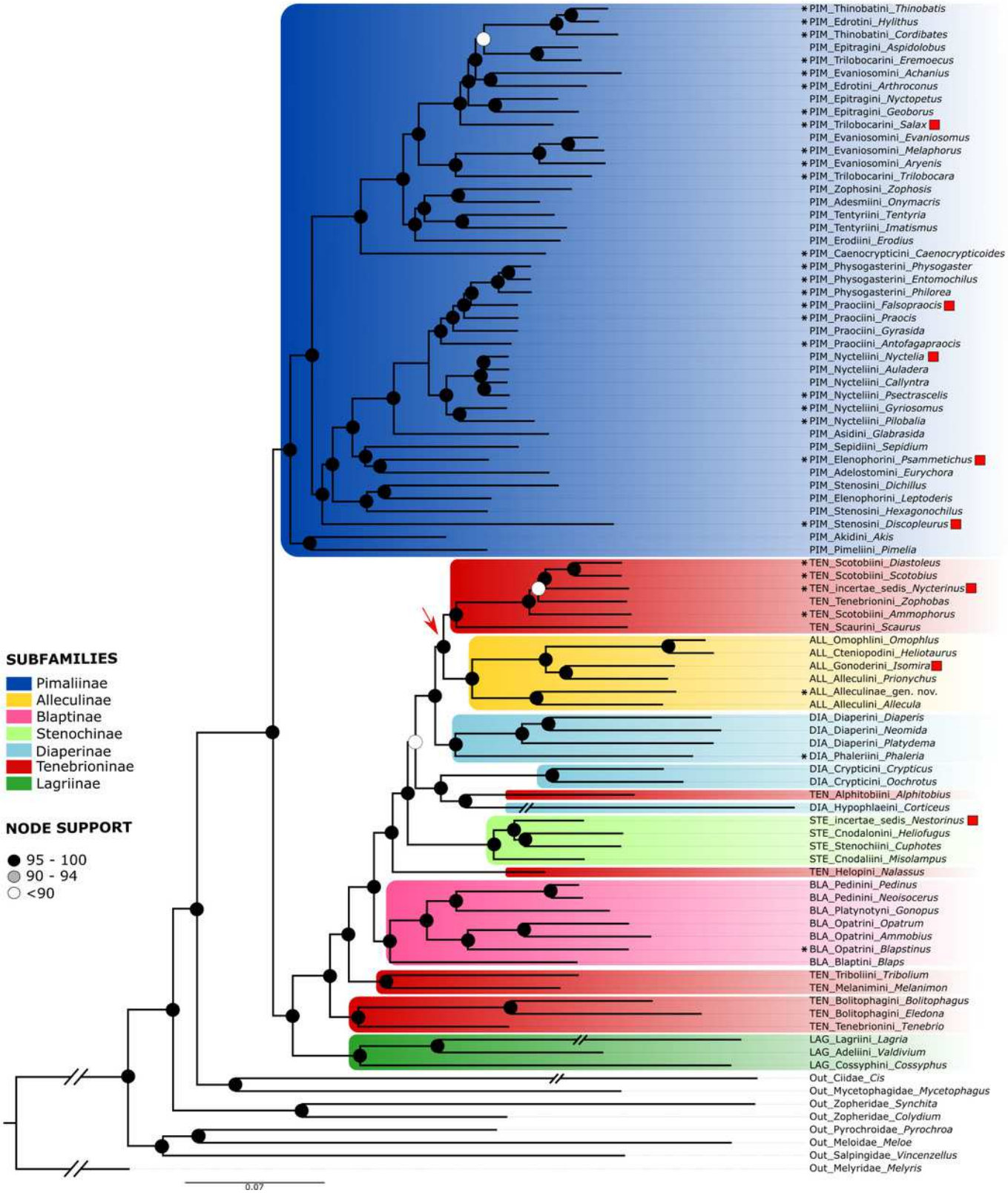
SUBFAMILIES
Pimaliinae
Alleculinae
Blaptinae
Stenochinae
Diaperinae
Tenebrioninae
Lagriinae
NODE SUPPORT
95 - 100
90 - 94
<90
0.07
*PIM_Thinobatini_Thinobatis
*PIM_Edrotini_Hylithus
*PIM_Thinobatini_Cordibates
PIM_Epitragini_Aspidolobus
*PIM_Trilobocarini_Eremoecus
*PIM_Evaniosomini_Achanius
*PIM_Edrotini_Arthroconus
PIM_Epitragini_Nyctopetus
*PIM_Epitragini_Geoborus
*PIM_Trilobocarini_Salax
PIM_Evaniosomini_Evaniosomus
*PIM_Evaniosomini_Melaphorus
*PIM_Evaniosomini_Aryenis
*PIM_Trilobocarini_Trilobocara
PIM_Zophosini_Zophosis
PIM_Adesmiini_Onymacris
PIM_Tentyriini_Tentyria
PIM_Tentyriini_Imatismus
PIM_Erodiini_Erodius
*PIM_Caenocrypticini_Caenocrypticoides
*PIM_Physogasterini_Physogaster
*PIM_Physogasterini_Entomochilus
*PIM_Physogasterini_Philorea
*PIM_Praociini_Falsopraocis
*PIM_Praociini_Praocis
PIM_Praociini_Gyrasida
*PIM_Praociini_Antofagapraocis
PIM_Nycteliini_Nyctelia
PIM_Nycteliini_Auladera
PIM_Nycteliini_Callyntra
*PIM_Nycteliini_Psectrascelis
*PIM_Nycteliini_Gyriosomus
*PIM_Nycteliini_Pilobalia
PIM_Asidini_Glabrasida
PIM_Sepidiini_Sepidium
*PIM_Elenophorini_Psammetichus
PIM_Adelostomini_Eurychora
PIM_Stenosini_Dichillus
PIM_Elenophorini_Leptoderis
PIM_Stenosini_Hexagonochilus
*PIM_Stenosini_Discopleurus
PIM_Akidini_Akis
PIM_Pimeliini_Pimelia
*TEN_Scotobiini_Diastoleus
*TEN_Scotobiini_Scotobius
*TEN_incertae_sedis_Nycterinus
TEN_Tenebrionini_Zophobas
*TEN_Scotobiini_Ammophorus
TEN_Scaurini_Scaurus
ALL_Omophlini_Omophlus
ALL_Cteniopodini_Heliotaurus
ALL_Gonoderini_Isomira
ALL_Alleculini_Prionychus
*ALL_Alleculinae_gen. nov.
ALL_Alleculini_Allecula
DIA_Diaperini_Diaperis
DIA_Diaperini_Neomida
DIA_Diaperini_Platydema
*DIA_Phaleriini_Phaleria
DIA_Crypticini_Crypticus
DIA_Crypticini_Oochrotus
TEN_Alphitobiini_Alphitobius
DIA_Hypophlaeini_Corticeus
STE_incertae_sedis_Nestorinus
STE_Cnodalonini_Heliofugus
STE_Stenochiini_Cuphotes
STE_Cnodaliini_Misolampus
TEN_Helopini_Nalassus
BLA_Pedinini_Pedinus
BLA_Pedinini_Neoisocerus
BLA_Platynotyni_Gonopus
BLA_Opatrini_Opatrum
BLA_Opatrini_Ammobius
*BLA_Opatrini_Blapstinus
BLA_Blaptini_Blaps
TEN_Triboliini_Tribolium
TEN_Melanimini_Melanimon
TEN_Bolitophagini_Bolitophagus
TEN_Bolitophagini_Eledona
TEN_Tenebrionini_Tenebrio
LAG_Lagriini_Lagria
LAG_Adeliini_Valdivium
LAG_Cossyphini_Cossyphus
Out_Ciidae_Cis
Out_Mycetophagidae_Mycetophagus
Out_Zopheridae_Synchita
Out_Zopheridae_Colydium
Out_Pyrochroidae_Pyrochroa
Out_Meloidae_Meloe
Out_Salpingidae_Vincenzellus
Out_Melyridae_Melyris

Table 1(on next page)

Taxa analyzed in phylogenetic reconstructions

List of Tenebrionidae and outgroup taxa (bold letters) analysed in this study, including statistics of assemblies after filtering. N50, the largest contigs size at which 50% of bases are contained in contigs of at least this length; BUSCO, Benchmarking Universal Single-Copy Orthologs. TSA, Transcriptome Shotgun Assembly accession number.

Species	Subfamily	Tribe	Country	N50	BUSCO[1]	TSA
Achanius piceus	Pimeliinae	Evaniosomini	Chile[¥]	3106	96.2%	pending
Akis trilineata	Pimeliinae	Akidini	Italy	2668	98.0%	pending
Allecula morio	Alleculinae	Alleculini	Germany	2724	97.5%	pending
Alleculinae gen. n.*	Alleculinae	?	Chile[¥]	1932	96.4%	pending
Alphitobius diaperinus	Tenebrioninae	Alphitobiini	Lab breeding	2028	97.0%	pending
Ammobius rufus	Blaptinae	Opatrini	Portugal	1828	96.8%	pending
Ammophorus cf. *peruvianus*	Tenebrioninae	Scotobiini	Chile[¥]	2042	96.8%	pending
Antofagapraocis brevipilis	Pimeliinae	Praociini	Chile [¥]	1954	91.8%	pending
Arthroconus sp.	Pimeliinae	Edrotini	Chile [¥]	1489	89.9%	pending
Aryenis unicolor	Pimeliinae	Evaniosomini	Chile [¥]	1978	92.0%	pending
Aspidolobus penai	Pimeliinae	Epitragini	Chile	1903	91.4%	pending
Auladera rugicollis	Pimeliinae	Nycteliini	Chile	1813	86.4%	pending
Blaps gibba	Blaptinae	Blaptini	Italy	2401	97.8%	pending
Blapstinus holosericeus	Blaptinae	Opatrini	Chile [¥]	2169	92.1%	pending
Bolitophagus reticulatus	Tenebrioninae	Bolitophagini	Germany	2937	95.3%	pending
Caenocrypticoides sp.*	Pimeliinae	Caenocrypticini	Chile [¥]	2373	98.4%	pending
Callyntra unicosta	Pimeliinae	Nycteliini	Chile	1782	86.2%	pending
***Cis* sp.**	Ciidae		Germany	2415	97.9%	pending
Colydium elongatum	Zopheridae		Germany	1798	96.4%	pending
Cordibates chilensis	Pimeliinae	Thinobatini	Chile [¥]	2296	95.5%	pending
Corticeus unicolor	Diaperinae	Hypophlaeini	Germany	2117	96.3%	pending
Cossyphus hoffmannseggi	Lagriinae	Cossyphini	Portugal	1935	97.4%	pending
Crypticus quisquilius	Diaperinae	Crypticini	Germany	2071	96.1%	pending
Cuphotes mercurius	Stenochiinae	Stenochiini	Chile	1955	90.4%	pending
Diaperis boleti	Diaperinae	Diaperini	Germany	2181	96.5%	pending
Diastoleus costalenis	Tenebrioninae	Scotobiini	Chile [¥]	2000	95.9%	pending
Dichillus subcostatus	Pimeliinae	Stenosini	Portugal	1778	97.0%	pending
Discopleurus sp.*	Pimeliinae	Stenosini	Chile [¥]	1561	95.6%	pending
Eledona agricola	Tenebrioninae	Bolitophagini	Germany	2443	97.0%	pending
Entomochilus rugosus	Pimeliinae	Physogasterini	Chile [¥]	2107	83.9%	pending
Eremoecus sp.	Pimeliinae	Trilobocarini	Chile [¥]	2081	95.0%	pending
Erodius goryi obtusus	Pimeliinae	Erodiini	Portugal	2199	96.1%	pending
Eurychora sp.	Pimeliinae	Adelostomini	Namibia	1674	91.0%	pending
Evaniosomus sp.	Pimeliinae	Evaniosomini	Peru	1649	87.8%	pending
Falsopraocis australis	Pimeliinae	Praociini	Chile	2388	93.4%	pending
Geoborus rugipennis	Pimeliinae	Epitragini	Chile [¥]	2522	93.6%	pending
Glabrasida punctipennis marseuli	Pimeliinae	Asidini	Portugal	1937	97.3%	pending
Gonopus sp.	Blaptinae	Platynotini	Namibia	1861	93.8%	pending
Gyrasida camilae	Pimeliinae	Praociini	Chile	1978	96.3%	pending
Gyriosomus curtisi	Pimeliinae	Nycteliini	Chile [¥]	2279	93.2%	pending
Heliofugus sp.	Stenochiinae	Cnodalonini	Chile	2063	97.6%	pending
Heliotaurus ruficollis	Alleculinae	Cteniopodini	Portugal	1877	85.1%	pending
Hexagonochilus	Pimeliinae	Stenosini	Chile	1988	96.2%	pending

tuberculatus						
Hylithus cf. *tentyroides*	Pimeliinae	Edrotini	Chile ¥	1971	94.3%	pending
Imatismus sp.	Pimeliinae	Tentyriini	Namibia	1305	85.4%	pending
Isomira semiflava	Alleculinae	Gonoderini	Germany	2385	90.0%	pending
Lagria sp.	Lagriinae	Lagriini	South Africa	2023	96.1%	pending
Leptoderis collaris	Pimeliinae	Elenophorini	Spain	2697	95.0%	pending
Melanimon tibialis	Tenebrioninae	Melanimini	Portugal	2516	97.4%	pending
Melaphorus elegans	Pimeliinae	Evaniosomini	Chile ¥	1224	77.3%	pending
Meloe proscarabaeus	Meloidae		Germany	2036	95.4%	pending
***Melyris* sp.**	Melyridae		South Africa	1372	85.9%	pending
Misolampus gibbulus	Stenochiinae	Cnodalonini	Portugal	2211	96.5%	pending
Mycetophagus quadripustulatus	Mycetophagidae		Germany	2623	98.6%	pending
Nalassus laevioctostriatus	Tenebrioninae	Helopini	Germany	2007	96.6%	pending
Neoisocerus ferrugineus	Blaptinae	Pedinini	Portugal	1845	86.7%	pending
*Neomida bicornis***	Diaperinae	Di[illegible]ini	USA	n/a	n/a	GDMA01.1
Nestorinus sp.*	Stenochiinae	?	Chile	1924	94.6%	pending
Nyctelia varipes	Pimeliinae	Nycteliini	Chile	1636	88.0%	pending
Nycterinus atacamensis	Tenebrioninae	*incertae sedis*	Chile ¥	2338	70.6%	pending
Nyctopetus tenebrioides	Pimeliinae	Epitragini	Chile	2453	96.9%	pending
Omophlus lepturoides	Alleculinae	Omophlini	Germany	2885	96.7%	pending
Onymacris rugatipennis	Pimeliinae	Adesmiini	Namibia	2079	96.2%	pending
Oochrotus unicolor	Diaperinae	Crypticini	Portugal	1848	96.6%	pending
Opatrum sabulosum	Blaptinae	Opatrini	Germany	1947	96.6%	pending
Pedinus sp.	Blaptinae	Pedinini	Portugal	1986	95.3%	pending
Phaleria gayi	Diaperinae	Phaleriini	Chile ¥	1840	96.0%	pending
Philorea sp.	Pimeliinae	Physogasterini	Chile ¥	1969	92.6%	pending
Physogaster sp.*	Pimeliinae	Physogasterini	Chile ¥	2290	83.5%	pending
Pilobalia sp.*	Pimeliinae	Nycteliini	Chile ¥	2041	96.3%	pending
Pimelia rugulosa	Pimeliinae	Pimeliini	Italy	1440	90.7%	pending
Platydema violaceum	Diaperinae	Diaperini	Germany	2405	97.3%	pending
Praocis sp.	Pimeliinae	Praociini	Chile ¥	2123	91.5%	pending
Prionychus melanarius	Alleculinae	Alleculini	Germany	2187	96.1%	pending
Psammetichus pilipes	Pimeliinae	Elenophorini	Chile ¥	2029	95.5%	pending
Psectrascelis confinis	Pimeliinae	Nycteliini	Chile ¥	2414	97.6%	pending
Pyrochroa serraticornis	Pyrochroidae		Germany	2805	95.2%	pending
Salax lacordairei	Pimeliinae	Trilobocarini	Chile ¥	2179	95.1%	pending
Scaurus uncinus	Tenebrioninae	Scaurini	Portugal	2003	95.7%	pending
Scotobius brevipes	Tenebrioninae	Scotobiini	Chile ¥	2108	87.3%	pending
Sepidium bidentatum	Pimeliinae	Sepidiini	Portugal	1780	96.3%	pending
Synchita undata	Zopheridae		Germany	2632	96.1%	pending
Tenebrio molitor ***	Tenebrioninae	Tenebrionini	Lab breeding	n/a	n/a	GIPG00000000
Tentyria cf. *laevigata*	Pimeliinae	Tentyriini	Italy	2153	97.3%	Pending
Thinobatis calderana	Pimeliinae	Thinobatini	Chile ¥	2415	96.5%	Pending
Tribolium castaneum	Tenebrioninae	Triboliini	Lab breeding	n/a	n/a	GCA_000002335.3

Trilobocara ciliatus	Pimeliinae	Trilobocarini	Chile ¥	1818	90.1%	Pending
Valdivium sp.*	Lagriinae	Adeliini	Chile	1592	95.8%	pending
Vincenzellus ruficollis	Salpingidae		Germany	3049	98.1%	pending
*Zophobas atratus****	Tenebrioninae	Tenebrionini	Lab breeding	n/a	n/a	GIPJ00000000
Zophosis sp.	Pimeliinae	Zophosini	Namibia	1729	92.7%	pending

1

2 * undescribed species
3 ** transcriptome data from McKenna et al. (2019)
4 *** transcriptome data from Marciniak et al. (2022);
5 ¥ species from Atacama Desert
6 [1] Insecta database (http://busco.ezlab.org/v2/datasets/insecta_odb9.tar.gz)