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A molecular phylogeny of chafers revisits the polyphyly of Tanyproctini (Scarabaeidae, Melolonthinae)

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Abstract

Tanyproctini (Melolonthinae) is a large group of chafers within the pleurostict Scarabaeidae that shows an enormous morphological diversity and variation. However, their morphology based definition appears to be mainly based on presumably plesiomorphic characters. Here, we investigate the phylogeny of this interesting lineage with a three-gene data set using partial gene sequences of 28S rRNA, cytochrome c oxidase I (cox1) and 16S rRNA (rrnL). Our data set comprised 191 species of all major lineages of pleurostict scarabs. Combined analyses of the 2,070 base pairs alignment with maximum-likelihood and Bayesian tree inference always recovered Tanyproctini to be highly polyphyletic. Tests of an alternative topology with constrained monophyly of Tanyproctini using CONSEL and IQ-TREE were not found to be more likely than the unconstrained tree topology. Instead, Tanyproctini was split into six independent lineages under the current taxon sampling that were scattered throughout diverse parts of the pleurostict tree. The fact that numerous smaller chafer lineages exist beside several evolutionary successful and large lineages, highlights the complexity of the pleurosticts' evolutionary history. The resulting tree topologies imply the need for a thorough revision of tribal classification within Melolonthinae lineages to accommodate the polyphyly of Tanyproctini. However, a revision of classification would be premature due to low support of most relevant branches, instable tree topologies among different tree searches, and due to a still very incomplete representation of Tanyproctini lineages.

1 | INTRODUCTION

Melolonthinae belong to phytophagous scarabs (Coleoptera: Scarabaeidae: Pleurosticti), a very diverse group of some 25,000 described species of beetles (Scholtz & Grebennikov, 2016) representing more than two-thirds of all species in the superfamily Scarabaeoidea. Pleurosticts were early recognized as a genealogical unit (Erichson, 1847), and their monophyly is supported by a number of distinct morphological synapomorphies (Balthasar, 1963; Browne & Scholtz, 1998; Ritcher, 1958). They are usually subdivided into four major subfamilies including Dynastinae, Rutelinae, Melolonthinae and Cetoniinae, plus several other small groups (Smith, 2006). Most species are highly polyphagous, with adults generally feeding on leaves, flowers or pollen of a wide range of plant taxa, and larvae primarily feeding on soil humus, living

roots or decaying wood. Therefore, their tremendous diversity cannot be explained by insect–host plant co-diversification (Ehrlich & Raven, 1964; Farrell, 1998; Mitter, Farrell, & Futuyma, 1991). Alternative hypotheses are needed that may explain their successful diversification (Eberle, Myburgh, & Ahrens, 2014). Due to the general species richness of major extant pleurostict scarab lineages, they are relatively easy sampled for molecular studies and thus already well covered by previous phylogenetic and evolutionary studies (Ahrens, Schwarzer, & Vogler, 2014; Ahrens, Scott, & Vogler, 2011; Ahrens & Vogler, 2008; Gunter, Weir, Slipinski, Bocak, & Cameron, 2016; Hunt et al., 2007; Šípek, Fabrizi, Eberle, & Ahrens, 2016). Several smaller lineages, however, are insufficiently represented and consequently poorly defined. Yet, the systematic knowledge on these smaller lineages (i.e., their presence in a phylogenetic hypothesis) is crucial for a comprehensive understanding of the historical and causal background of scarab diversification.

Among these smaller lineages is the melolonthine tribe Tanyproctini (formerly Pachydemini, see Bouchard et al., 2011; Smith & Mondaca, 2016), a species-rich but poorly studied taxon for which Lacroix (2007) listed altogether 119 genera and 564 valid species. They are known from all major zoogeographical regions except Australia but their distribution is very disjunct. The majority of taxa are afrotropic and holarctic, the latter limited to western North America and the southern Palearctic. In the Neotropics, the tribe Tanyproctini is represented by 18 genera and 33 species that are restricted to the temperate region with the greatest diversity in Argentina (Sanmartín & Martín-Piera, 2003; Smith & Mondaca, 2016). They are also more abundant (both in number of species and genera) in the southeastern part of the Afrotropical region (Lacroix, 2000). These disjunct distributions, together with reduced geographic ranges of most species (females are usually flightless) (Sanmartín & Martín-Piera, 2003), make the lineage especially interesting for the study of evolution of herbivore scarabs.

Tanyproctini show an enormous morphological diversity and variation. Following the morphology based definition of Lacroix (2000, 2007), the tribe appears to be defined mainly by presumably plesiomorphic characters (Ahrens, 2006). Therefore, a test of their monophyly and investigation of their systematic position is essential for a deeper understanding of phytophagous scarab evolution (Ahrens et al., 2014; Gunter et al., 2016). One consequence of the lack of reliable characters for their classification is the tendency of either monotypic or species-poor genera (Sanmartín & Martín-Piera, 2003). About 62% of the palearctic genera of Tanyproctini contain only one species (Lacroix, 2000). Most of the monotypic genera have been erected to accommodate single species that differ from traditional genera by a conspicuous autapomorphy. Some early phylogenetic studies based on morphology (Sanmartín & Martín-Piera, 2003) and 28S rDNA (Ocampo, Ruiz-Manzanos, & Marvaldi, 2010) revealed evidence that might indicate polyphyly of the species so far included in the tribe Tanyproctini.

Here, we investigate the phylogeny of this interesting lineage based on a three-gene data set that was steadily extended in the course of previous investigations of pleurosticts and that already proved its phylogenetic information content (Ahrens et al., 2014, 2011; Ahrens & Vogler, 2008; Gunter et al., 2016; Hunt et al., 2007; Šípek et al., 2016). In particular, we test the hypothesis of polyphyly of the group, by adding a significant number of Tanyproctini species sampled during the past years in field surveys, particularly in Europe and southern Africa.

2 | MATERIAL AND METHODS

2.1 | Sampling and molecular lab procedures

The present study extends the taxon sampling of previous molecular phylogenies of pleurostict scarab chafers (Ahrens & Vogler, 2008; Eberle et al., 2014; Liu et al., 2012; Šípek et al., 2016) with particular regard on species so far assigned to the tribe Tanyproctini (Supporting Information Table S1 and Figure S1). Our data set comprised 191 species of all major lineages of pleurostict scarabs (Supporting Information Table S1), including 45 newly sequenced taxa. Specimen collection, preservation and DNA extraction followed Ahrens and Vogler (2008). Vouchers are deposited in the collections of the Zoological Research Museum A. Koenig, Bonn (ZFMK). Two mitochondrial markers, the 3‘ end of cytochrome oxidase subunit 1 (*cox1*) and 16S ribosomal DNA (*rrnL*), and a fragment of nuclear 28S rDNA, containing the variable domains D3-D6 were used in our analysis. New samples were collected during fieldwork in South Africa which was enabled by the following collection permits: Eastern Cape (Permit No.: WRO 122/07WR and WRO123/07WR), Gauteng (Permit No.: CPF6 1281), Limpopo (Permit No.: CPM-006-00001), Mpumalangma (Permit No.: MPN-2009-11-20-1232) and Kwazulu-Natal (Permit Nos OP3752/2009, 1272/2007, 3620/2006).

Newly sequenced specimens were preserved in 96% ethanol. DNA was extracted from the left mid-leg and from thoracic flight muscles of ethanol-preserved specimens with *Qiagen® DNeasy Blood & Tissue Kits* using standard protocols. Subsequently, the genitalia were glued on a card and dry mounted on the same pin as the specimen. The mitochondrial markers and nuclear DNA fragments, as described above, were amplified with polymerase chain reaction. *Qiagen® Multiplex PCR Kits* were used with primers stevPat and stevJerry for *cox1* (Timmermans et al., 2010), 16Sar and 16sB2 for *rrnL* (Simon et al., 1994), and FF and DD (Monaghan, Inward, Hunt, & Vogler, 2007) for 28S. Forward and reverse strands were sequenced by Macrogen (Seoul, South Korea) using the same primers. Sequences were manually edited in Geneious 7.1.8.

2.2 | Multiple sequence alignment and phylogenetic inference

Since multiple sequence alignment can be problematic for large datasets, especially when markers with highly variable regions like *rrnL* are included, we employed the divide-and-conquer realignment technique implemented in SATé-II (version 2.2.7, Liu et al., 2012). This method simultaneously estimates a phylogenetic tree and the alignment in multiple iterations and can lead to great improvements in hard-to-align data sets by deconstructing the alignment to smaller, closely related subsets of sequences (subproblems), which are separately aligned and subsequently merged. We ran 10 iterations on the multilocus data set, aligning subproblems with a maximum size of 100 individuals with MAFFT (version 7.299b, Katoh & Toh, 2008, 2010). Subproblems were generated by the centroid strategy and remerged with Muscle (version 3.7, Edgar, 2004a, 2004b). The simultaneous tree estimation was done with RAxML (version 8.2.9, Stamatakis, 2014).

We used Bayesian inference to reconstruct the tree topology with MrBayes (version 3.2, Huelsenbeck & Ronquist, 2001; Ronquist et al., 2012). The data was partitioned (Brandley, Schmitz, & Reeder, 2005; Nylander, Ronquist, Huelsenbeck, & Nieves-Aldrey, 2004) by *rrnL*, 28S and three codon positions of *cox1*. Tree searches were conducted for 5×10^7 MCMC generations, using a random starting tree and two runs of three heated and one cold Markov chain (heating of 0.1). Chains were sampled every 5,000 generations and 10% of generations were discarded as burn-in. Tracer 1.6 (Rambaut, Suchard, Xie, & Drummond, 2014) and RWTY (version 1.0.1; Wilgenbusch, Warren, & Swofford, 2004; Warren, Geneva, & Lanfear, 2017) were used to assess the convergence of runs. Branch supports were reported as posterior probabilities. Alternatively, phylogenetic relationships were also inferred using maximum likelihood (ML) in RAxML (version 8.2.8, Stamatakis, 2014). The combined matrix was partitioned for the three markers and the tree was estimated under the GTR+CAT model (Stamatakis, 2006) with final optimization under the GTR+Γ model. Base frequencies were estimated for each partition.

Branch support of ML trees was assessed by (a) the non-parametric Shimodaira–Hasegawa-like implementation (SHL, Guindon et al., 2010) of the approximate likelihood-ratio test (aLRT, Anisimova & Gascuel, 2006; Anisimova, Gil, Dufayard, Dessimoz, & Gascuel, 2011; Guindon et al., 2010), (b) 100 standard bootstrap replicates (SBS), and (c) 1,000 rapid bootstrap replicates (RBS) (Stamatakis, Hoover, & Rougemont, 2008). Requirements of SBS (e.g., site independence) are rarely met by empirical data (Pease, Brown, Walker, Hinchliff, & Smith, 2018). RBS, SH-like aLRT supports and, in particular, SBS are known to underestimate the true probability of a clade (Minh, Nguyen, & Haeseler, 2013). We adopt a conservative approach by considering branches with SHL-values >85 as strongly supported (Anisimova et al., 2011; Guindon et al., 2010; Pyron, 2014; Pyron & Wiens, 2011). Standard and rapid bootstrap values were considered strong support above 80 which roughly corresponds to a probability of 0.95 to be correct (Minh et al., 2013).

An alternative tree hypothesis with a single monophyletic Tanyproctini lineage was inferred in a constraint RAxML analysis and evaluated against the unconstrained topology using the site bootstrapping procedure implemented in CONSEL (Shimodaira & Hasegawa, 2001). This software identifies the top-ranking topology for alternative tree hypotheses under the likelihood criterion and assesses the support for each topology; the programme calculates *p*-values for an approximately unbiased test (AU) and performs Bootstrap Probability (NP, BP and PP), Shimodaira–Hasegawa (SH) and weighted Shimodaira–Hasegawa (WSH) tests. We used the default scaling factors of 0.5–1.4, with 10,000 pseudoreplicates for each run. Individual site likelihoods used in the CONSEL analysis were calculated for competing topological hypothesis (constrained and unconstrained) using RAxML. Alternatively, we used more sophisticated implementations of the topology tests in IQ-Tree (http://www.iqtree.org/) (Nguyen, Schmidt, von Haeseler, & Minh, 2015) which in contrast to CONSEL are partition-aware and thus more appropriate. Slight discrepancies in AU-test p-values of CONSEL and IQ-TREE might also result from differing implementations strategies (ML estimates and least-squares estimates, respectively).

3 | RESULTS

3.1 | Phylogenetic inference

The phylogenetic relationships of main pleurostict lineages based on the alignment of 2,070 base pairs largely coincided between maximum likelihood (ML) and Bayesian (BY) tree inference (Figure 1, Supporting Information Figure S2). Furthermore, resulting topologies were widely congruent with previous hypotheses (Ahrens et al., 2014, 2011; Ahrens & Vogler, 2008; Šípek et al., 2016): The clade of southern world Melolonthinae was the earliest branch being sister to all other pleurosticts, with Sericini + Ablaberini being the next branching clade among these, and the stable monophyletic clade of Cetoniinae + Rutelinae (including Dynastinae as sister to Adoretini) being nested within other Melolonthine lineages. In contrast to Ahrens and Vogler (2008) and Eberle, Fabrizi, Lago, and Ahrens (2017), the specimens of South American Sericini (*Astaena* spp.) were not recovered within the monophyletic clade of Sericini but as sister to Sericini + Ablaberini. All major tribes resulted monophyletic in a similar way, except Macrodactylini and Valgini in the BY tree). However, there were slight variations of their relationship between previous studies and the present results (e.g., within "Melolonthini"; Macrodactylini vs. Euchirini). Interestingly, Orphninae, was

recovered again as "rogue lineage" within the pleurostict lineages as in previous studies (e.g., Ahrens et al., 2014; Šípek et al., 2016), although their sister-group relationship with Pleurosticti is quite robustly founded by morphology (Ahrens, 2006).

Here, in both tree searches, Tanyproctini resulted to be polyphyletic. The test of an alternative topology with constrained monophyly of Tanyproctini using CONSEL and IQ-TREE was not found to be more likely

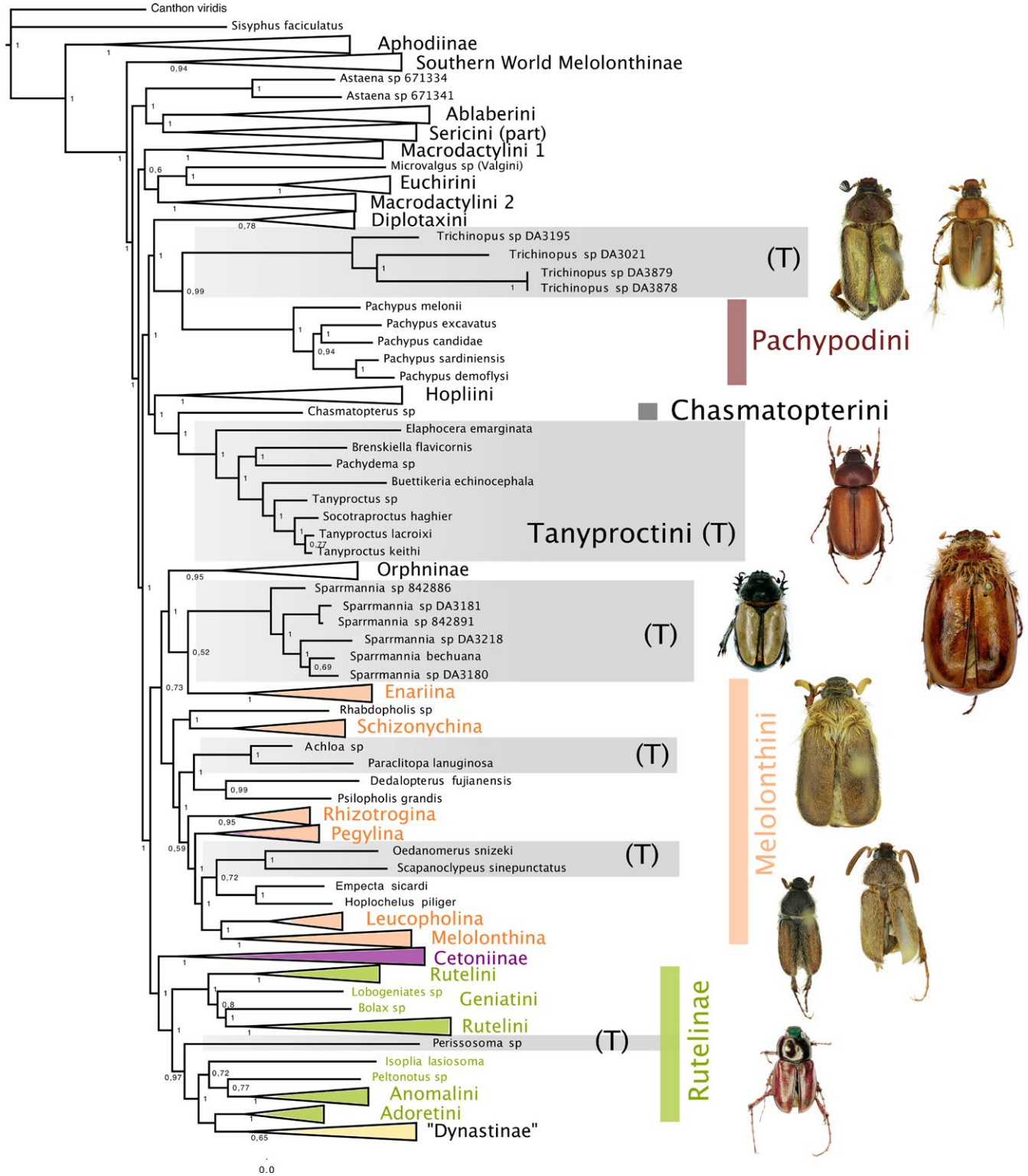

FIGURE 1 Bayesian majority rule consensus tree (BY) showing the phylogeny of Melolonthine chafers, with larger tribes that have been previously investigated not shown (see Supporting Information Figure S1 for details). Posterior probabilities above 0.5 are annotated close to the nodes [Colour figure can be viewed at wileyonlinelibrary.com]

than the unconstrained tree topology (Table 1). Instead, Tanyproctini was split into six independent lineages under the current taxon sampling. One included the type genus *Tanyproctus* and all Mediterranean species so far assigned to Tanyproctini. This clade is likely to represent truly the subtribe Tanyproctina (Bouchard et al., 2011) (referred to in the following as "Palearctic Tanyproctina"). It was consistently found in all tree searches to be sister of Chasmatopterini, and both together either sister of Hopliini (Figure 1) or sister to the rest of pleurosticts (excluding Liparetrinae and Sericini + Ablaberini) (Figure 2). Another clade including the genus *Trichinopus* was in both trees found as sister of Pachypodini which were both together sister of Diplotaxini. In previous analysis Pachypodini was found to be sister of Rutelinae (incl. Dynastinae) (Ahrens et al., 2014) which, however, was in that former analysis only represented by a single species. Another separate larger clade comprised the genus *Sparrmannia*, and was in both analyses in two different positions: nested as sister to Enariina (Figure 1) or as sister to the clade Cetoniinae + Rutelinae. The position of the next isolated two Tanyproctini lineages was constant in both trees: one included *Achloa* and *Paraclitopa* being sister to *Dedalopterus* + *Psilopholis*, the other was composed of *Oedanomerus* and *Scapanoclypeus* being sister to *Empecta* + *Hoplochelus*. Finally, the last isolated "Tanyproctini lineage" included *Perissosoma* and was nested within Rutelinae at different positions (Figures 1 and 2).

Tests of alternative constrained topologies of a monophyletic Tanyproctini clade with CONSEL highly supported the unconstrained analysis (Table 1) and thus the hypothesis, that the tribe as currently defined is very likely polyphyletic. Results of tests with IQ-TREE were similar, however, less pronounced. Significance at the 0.05 level was still recovered for the approximately unbiased test (Shimodaira, 2002) and the bootstrap proportion test (Kishino & Hasegawa, 1989).

3.2 | Analysis without rogue taxa

Microvalgus and Orphninae resulted in either just the BY or in BY and ML analyses to be rogue taxa, that is, being in non-stable position to clades with supposedly unrelated lineages (Figures 1 and 2). If trees were inferred without these two taxa, the topology within major clades remained stable, however, the topology between some of these clades somewhat altered. Also, Melolonthini resulted monophyletic in the BY and the ML tree. However, *Sparrmannia* was never recovered in this clade. The *Sparrmannia* clade ended up in the BY tree as sister to Cetoniinae + Rutelinae (incl. Dynastinae), rather confirming the sister relationship with Enariini, while in the ML tree it was sister to Cetoniinae. The *Trichinopus* clade in the BY tree was associated with Euchirini (rather than with Pachypodini) both being nested within Macrodactylini (Figure S3). The Palearctic Tanyproctina clade was in the BY tree again associated with Hopliini, while in the ML-tree it was sister to the Macrodactylini clade 1. Macrodactylini clade 2 was sister to Euchirini (Supporting Information Figure S4). The position of *Perissosoma* sp. was not stable in any of the analyses, however, it was always placed within "Rutelinae", either as sister of "Dynastinae" (Supporting Information Figure S4), as sister of Adoretini, (Anomalini + Dynastinae) (Figure 1, Supporting Information Figure S3), or as sister of Rutelini + Geniatini (Figure 2).

4 | DISCUSSION

The resulting tree topologies would imply the need for a thorough revision of tribal classification within Melolonthinae lineages to accommodate the polyphyly of Tanyproctini. Compared to the first phylogenetic analysis of Tanyproctini, which were exclusively based on morphological traits (Sanmartín & Martín-Piera, 2003), this study used a much

TABLE 1 Test results on the monophyly of Tanyproctini from IQ-TREE and CONSEL. In both cases the unconstrained topology is preferable

	Δ*L*	*p*-AU	np	bp	*p*-KH	*p*-SH	*p*-WKH	*p*-WSH	c-ELW	pp
IQ-TREE										
Unconstrained	0.000	0.9493	—	0.9454	0.9422	1.0000	0.9422	0.9422	0.9453	—
Tanyproctini constrained to be monophyletic	67.830	0.0507	—	0.0546	0.0578	0.0578	0.0578	0.0578	0.0547	—
Consel										
Unconstrained	−112.6	0.994	0.993	0.993	0.993	0.993	0.993	0.993	—	1.000
Tanyproctini constrained to be monophyletic	112.6	0.006	0.007	0.007	0.007	0.007	0.007	0.007	—	1e−49

Note. bp, bootstrap proportion (Kishino & Hasegawa, 1989); c-ELW, expected likelihood weight (Strimmer & Rambaut, 2002); np, the bootstrap probability calculated from the multiscale bootstrap; p-AU, *p*-value of approximately unbiased (AU) test (Shimodaira, 2002); *p*-KH, *p*-value of one sided Kishino–Hasegawa test (1989); pp, Bayesian posterior probability calculated by the BIC approximation; *p*-SH, *p*-value of Shimodaira–Hasegawa test (Shimodaira & Hasegawa, 1999); *p*-WKH, *p*-value of weighted KH test; *p*-WSH, *p*-value of weighted SH test; Δ*L*, log*L* difference from the maximal logl in the set.

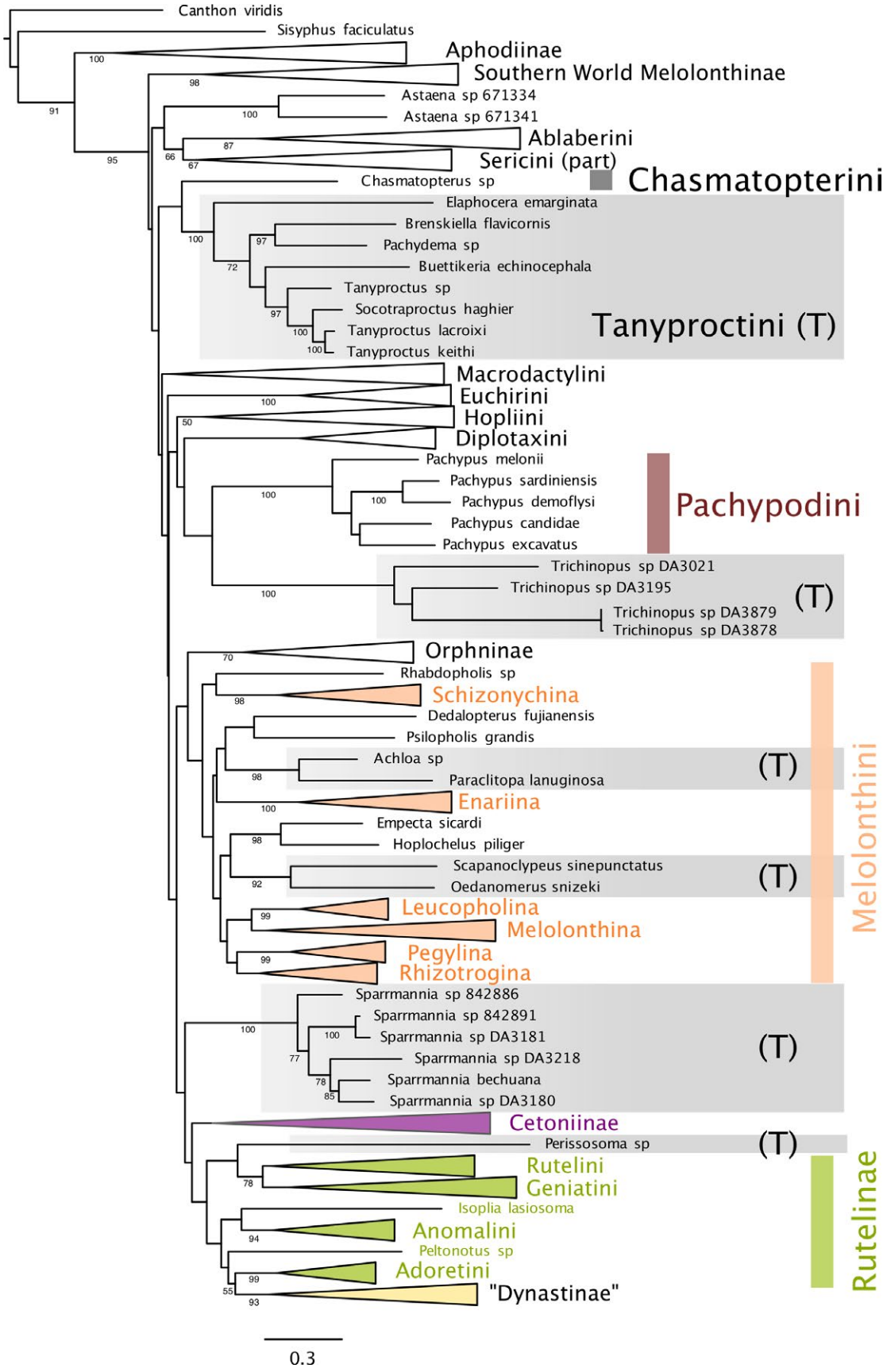

FIGURE 2 Best RAxML tree (ML) showing the phylogeny of Melolonthine chafers, with some lineages not shown (see Supporting Information Figure S2 for details). RBS node support is annotated close to branches [Colour figure can be viewed at wileyonlinelibrary.com]

more comprehensive and balanced sampling of pleurostic lineages. The former included only few non-Tanyproctini species, which hampers the evaluation of polyphyly: two species of Rhizotrogini and Melolonthini; their tree was rooted with a species of Sericini. Except one African (*Sparrmannia*) and one Nearctic (*Phobetus*) taxon, all Tanyproctini were from the Palearctic region, presumably related to our Palearctic Tanyproctina clade. While polyphyly of Tanyproctini was already evident from their study, its sampling was too limited to recognize the true extend of non-relatedness of lineages so far comprised in Tanyproctini.

Except Ocampo et al. (2010), all other molecular phylogenies so far published (Ahrens et al., 2014; Gunter et al., 2016; Šípek et al., 2016) neglected the tribe. Already Ocampo et al. (2010) found some evidence for the polyphyly of Tanyproctini, however, topologies resulted from analysis of a single gene and rather limited sampling. It only included three Neotropical Tanyproctini genera, of which one in the end nested close to Rutelinae. Our analysis represents in regard of sampled taxa and number of used loci a considerable step ahead towards the understanding of the phylogeny of melolonthine chafer lineages and their classification, in particular with respect to Tanyproctini. However, we feel that it would be premature to derive systematic and nomenclatural changes due to low support of many relevant branches, instable tree topologies among different tree search algorithms, and also due to still very incomplete representation of lineages so far included in Tanyproctini. The resulting tree hypotheses, however, revealed that the evolutionary history of herbivore scarab lineages (Ahrens et al., 2014) is by far more complex, and in consequence also their classification. Our results reveal the existence of numerous smaller lineages beside several diverse and evolutionary successful lineages. These smaller lineages did not develop very large species richness but survived in course of the evolutionary radiation of herbivore scarabs. The instable position of such isolated single lineages in different tree searches is a phenomenon which is often encountered in phylogenetic trees (Aberer, Krompass, & Stamatakis, 2013) and additional problems such as long branch attraction may occur (Bergsten, 2005).

Currently, Tanyproctini is classified into two subtribes, Macrophyllina Burmeister, 1855, and Tanyproctina Erichson, 1847 (Bouchard et al., 2011; Smith, 2006). While the first species-poor group is not represented in our current nor any of the previous phylogenetic analyses (and is not further discussed here), the latter includes six synonyms of family-group names, of which two correspond to two monophyletic clades recovered in all of our analyses: (a) "Achloidae Burmeister, 1855" represented here by *Achloa* + *Paraclitopa*; and (b) Sparrmannini Péringuey, 1904 represented here by the monophyletic clade of *Sparrmannia* species. The first was placed in all analyses within the clade of Melolonthini, and thus its synonymy with Tanyproctina/Tanyproctini needs to be revised. As above mentioned, the position of the clade representing Sparrmannini seems phylogenetically rather unstable and isolated what could be an argument to justify it as an independent tribe rather than being a synonym of Tanyproctina/Tanyproctini. Likewise, the isolated *Perissosoma* should be ranked as a separate Ruteline tribe rather than a member of Tanyproctini due to its repeatedly found placement within that lineage. This is in line with the opinion of Lacroix (2007) to classify it as a separate family-group entity close to Dynastinae which was based on presence of a few morphological key characters rather than on phylogenetic analysis. Similar cases are to be expected from many other taxa so far included in Tanyproctini, but not available for analyses here (e.g., *Neogutierrezia* Martínez, 1953; see Ocampo et al., 2010).

Our results also provide insight in regard to the currently contrasting classification schemes of Melolonthine chafers. Subtribes of Melolonthini as listed by Smith (2006) and Bouchard et al. (2011) are all ranked as tribes by Löbl and Löbl (2016). Although there is no objective criterion for ranking a specific lineage at the level of tribe or subtribe, monophyly of all included melolonthini subtribes (plus two clades of former Tanyproctini) as found here after rogue taxa were excluded (Supporting Information Figures S3 and S4) would not contradict their handling as a single tribe Melolonthini. The latter is not feasible when their non-monophyly is a likely hypothesis. Similarly, tree hypotheses elaborated from our current data set also contradict the recent proposal of Cherman and Morón (2014) to keep the names Melolonthidae and Cetoniidae for the two principle lineages of pleurostict chafers retrieved by Hunt et al. (2007) from the same three gene markers as used for this analysis. Other previous studies with more intensive sampling and the same markers did not confirm just two principle lineages but many more isolated melolonthine lineages and a sister relationship between Cetoniinae + Rutelinae (inclusive Dynastinae) (Ahrens et al., 2011; Ahrens & Vogler, 2008; Gunter et al., 2016; Šípek et al., 2016). Similarly, more extensive data sets also always found Cetoniinae nested within a clade comprising Melolonthinae and Rutelinae (incl. Dynastinae) (Timmermans et al., 2016; Zhang et al., 2018).

Despite the above-mentioned limitations of our analysis, results revealed some highly interesting insights into a major systematic group of pleurostict chafers and a significant need to further investigate in detail the scarab phylogeny in order to overcome problems of classification and inference of their evolutionary history. The outcome of this study appears to be furthermore highly relevant for pleurostict fossil classification, as the existence of many isolated and polyphyletic melolonthine lineages makes the assignment of fossils to clades difficult, which also hampers the inference of divergence times of the group. Therefore, on the task list for future research is 1) to significantly expand sampling in terms

of taxa and loci by transcriptomic or hybrid enrichment data (e.g., Mayer et al., 2016; Misof et al., 2014) in order to gain more robust tree topologies, but also 2) to map morphological traits onto trees in order to allow morphological diagnosis of confirmed systematic groups.

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CONFLICT OF INTEREST

We have no competing interests.

AUTHORS' CONTRIBUTIONS

JE and DA involved in article conception and design. JE, DA, DC, GS, EB, PS, RS, AB and DK involved in data acquisition, drafting, revising and approving the article. JE and DA involved in analysis and interpretation of data.

DATA ACCESSIBILITY

DNA sequences were stored at GenBank (Supporting Information Table S1).

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SUPPORTING INFORMATION

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