

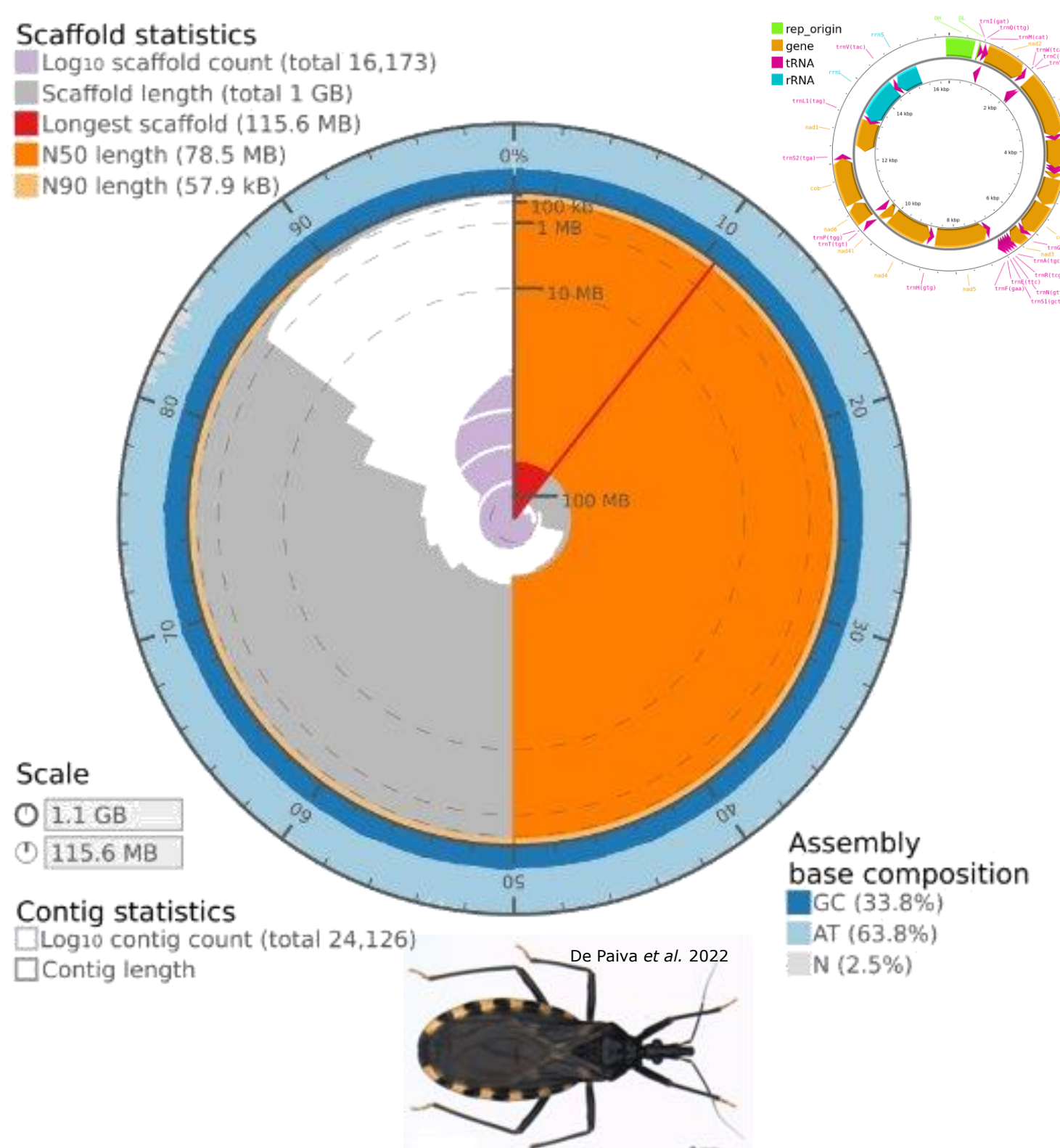


# A genomic basis for the transition to hematophagy in triatomines, vectors of Chagas disease

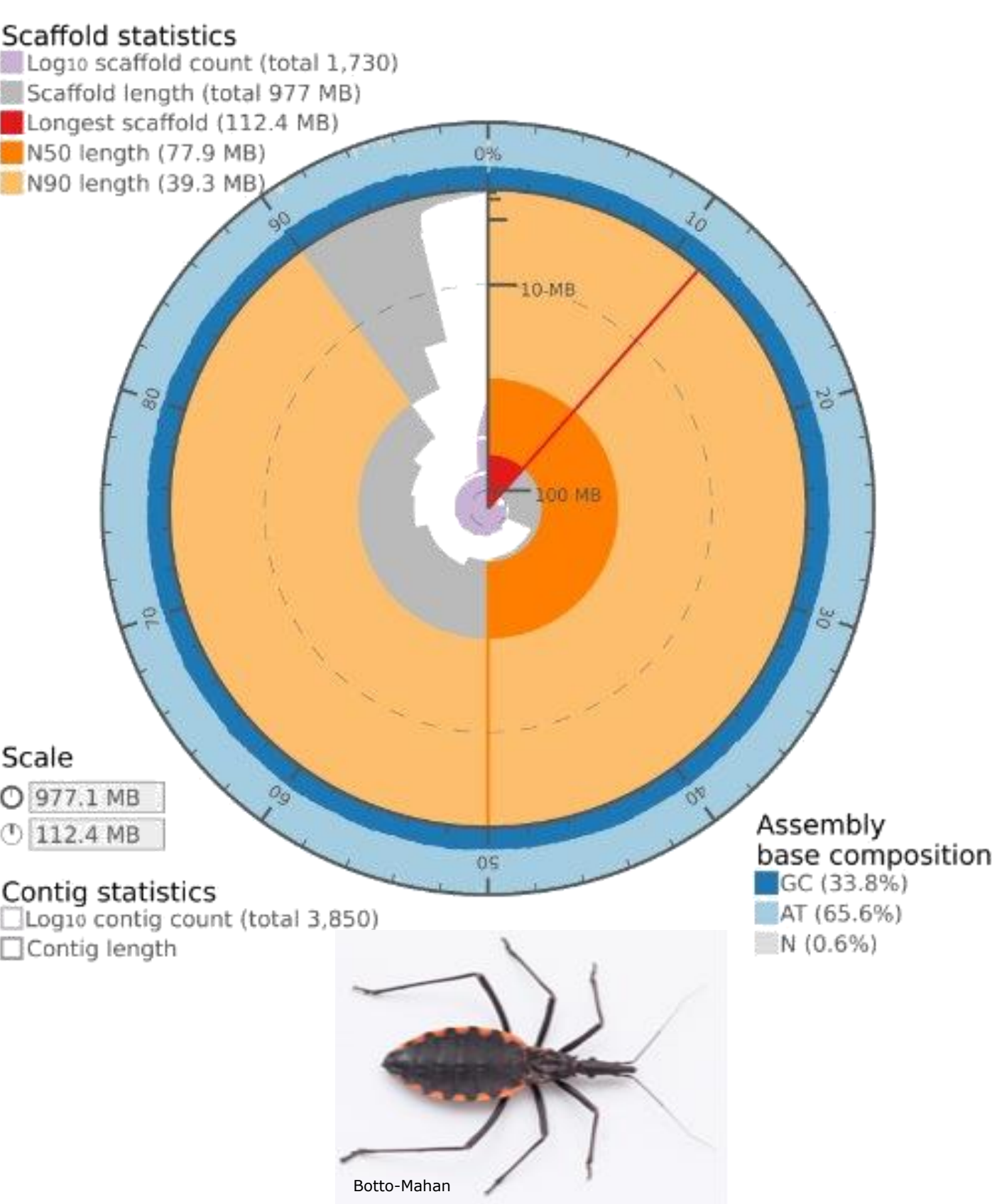
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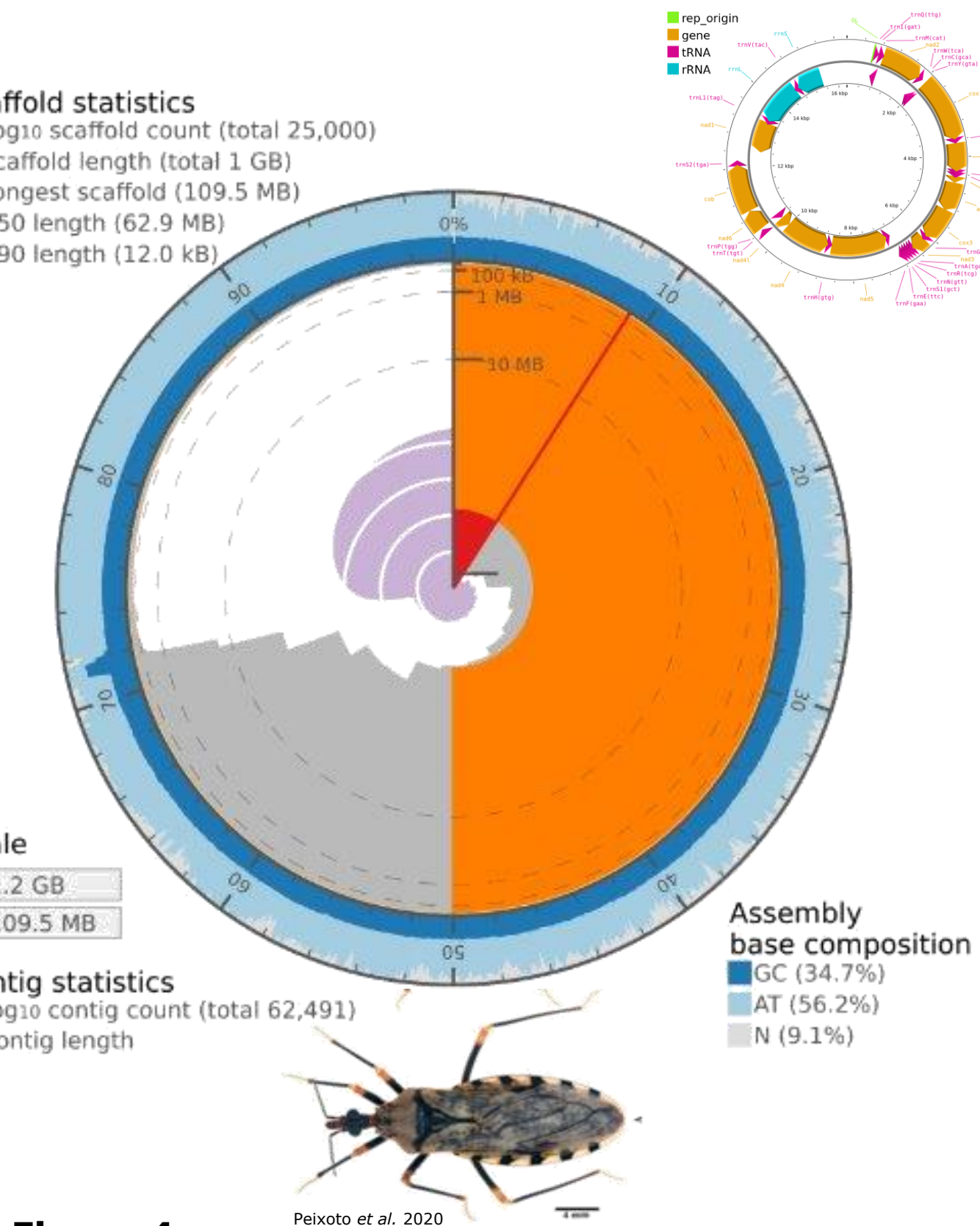
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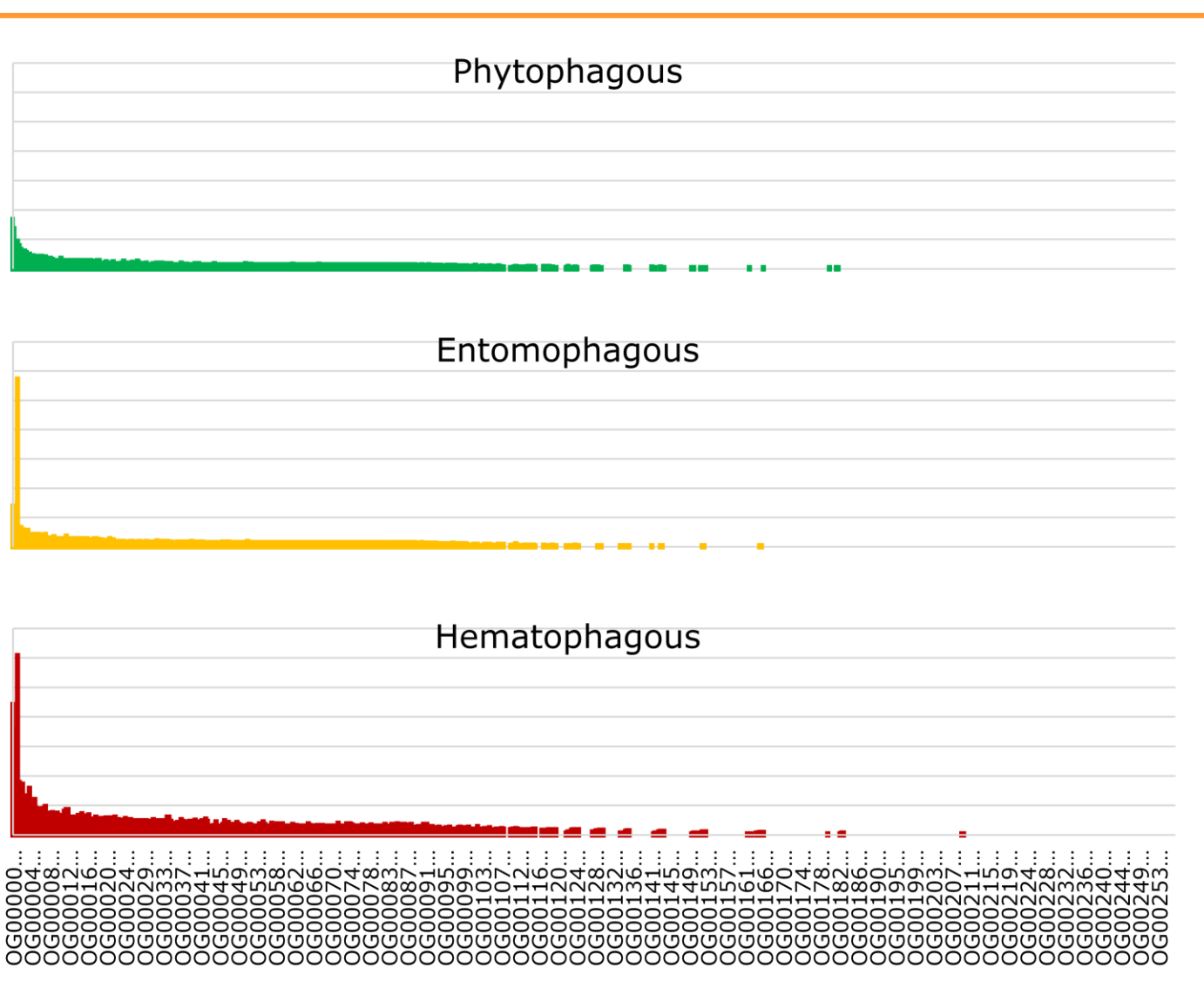
**Figure 2.** Tribe Triatomini: *Triatoma infestans* whole genome assembly graphical representation. Mitogenome representation (top right).



**Figure 3.** Tribe Triatomini: *Mepraia spinolai* whole genome assembly graphical representation. Mitogenome representation (top right).



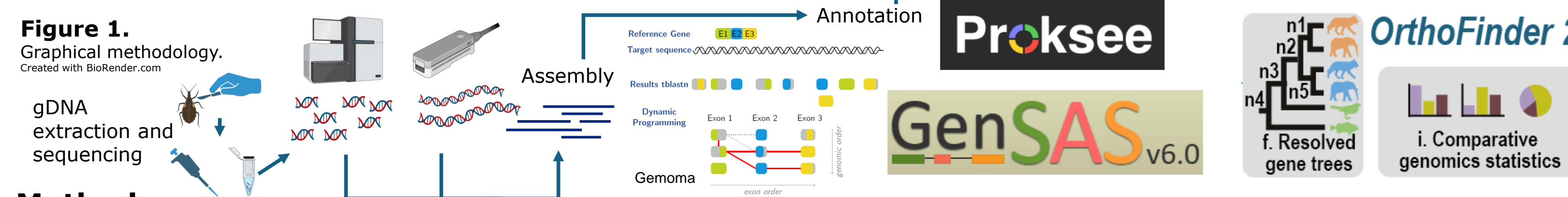
**Figure 4.** Tribe Triatomini: *Panstrongylus geniculatus* whole genome assembly graphical representation. Mitogenome representation (top right).



**Figure 13.** Ortholog histograms of *Belminus herrerii*, shared with phytophagous (top), entomophagous (middle) and hematophagous (bottom) species.

## Introduction

Chagas disease is the most important parasitosis on the American continent, with more than 300,000 new human cases and 12,000 deaths each year. New tools are required to accelerate the interruption of *Trypanosoma cruzi* domiciliary vectorial transmission by triatomines. Here, we provide multiple new genomic resources for triatomine species, including non-hematophagous predatory sister taxa, with the aim of elucidating the process of adaptation to blood feeding within Reduviidae (Hemiptera: Heteroptera).

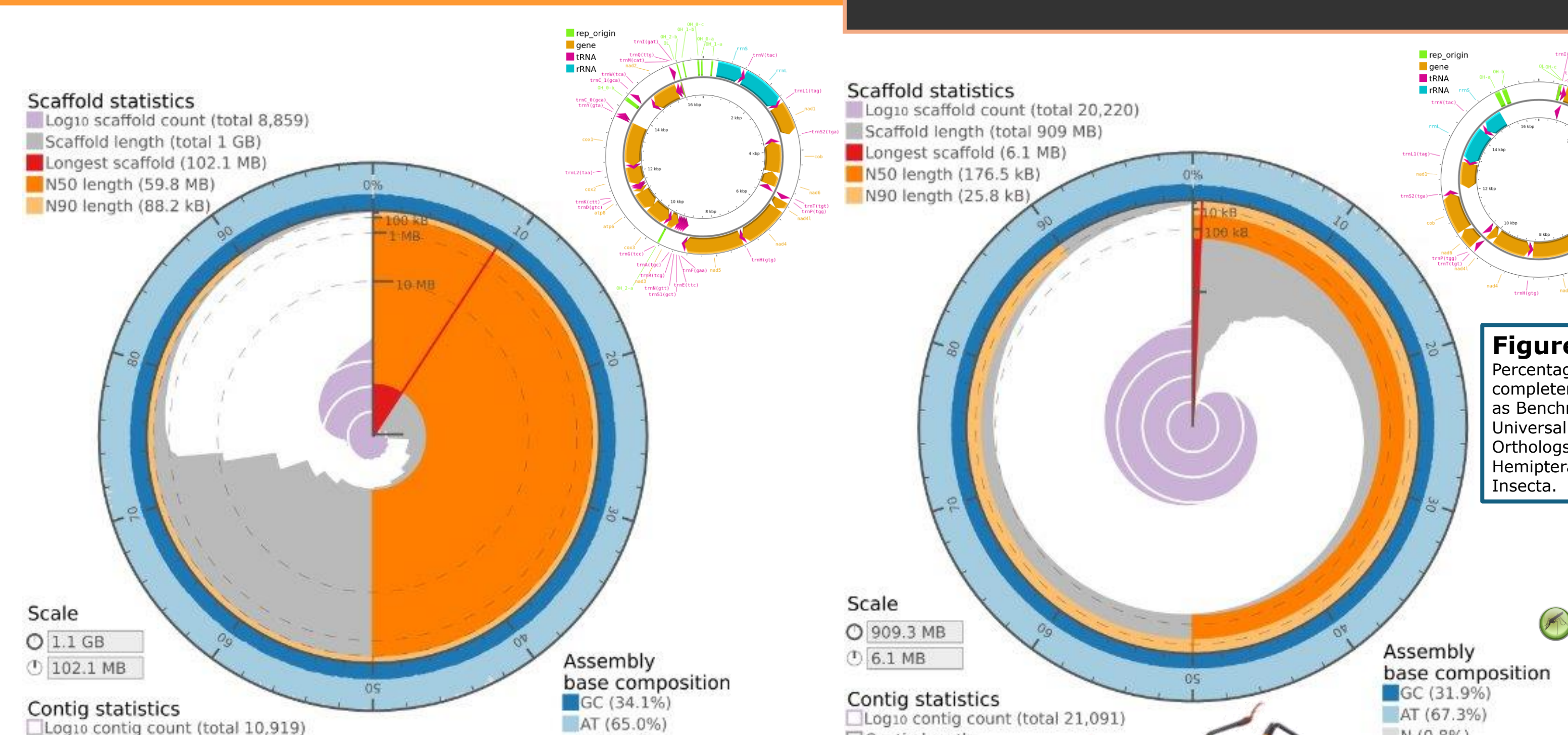
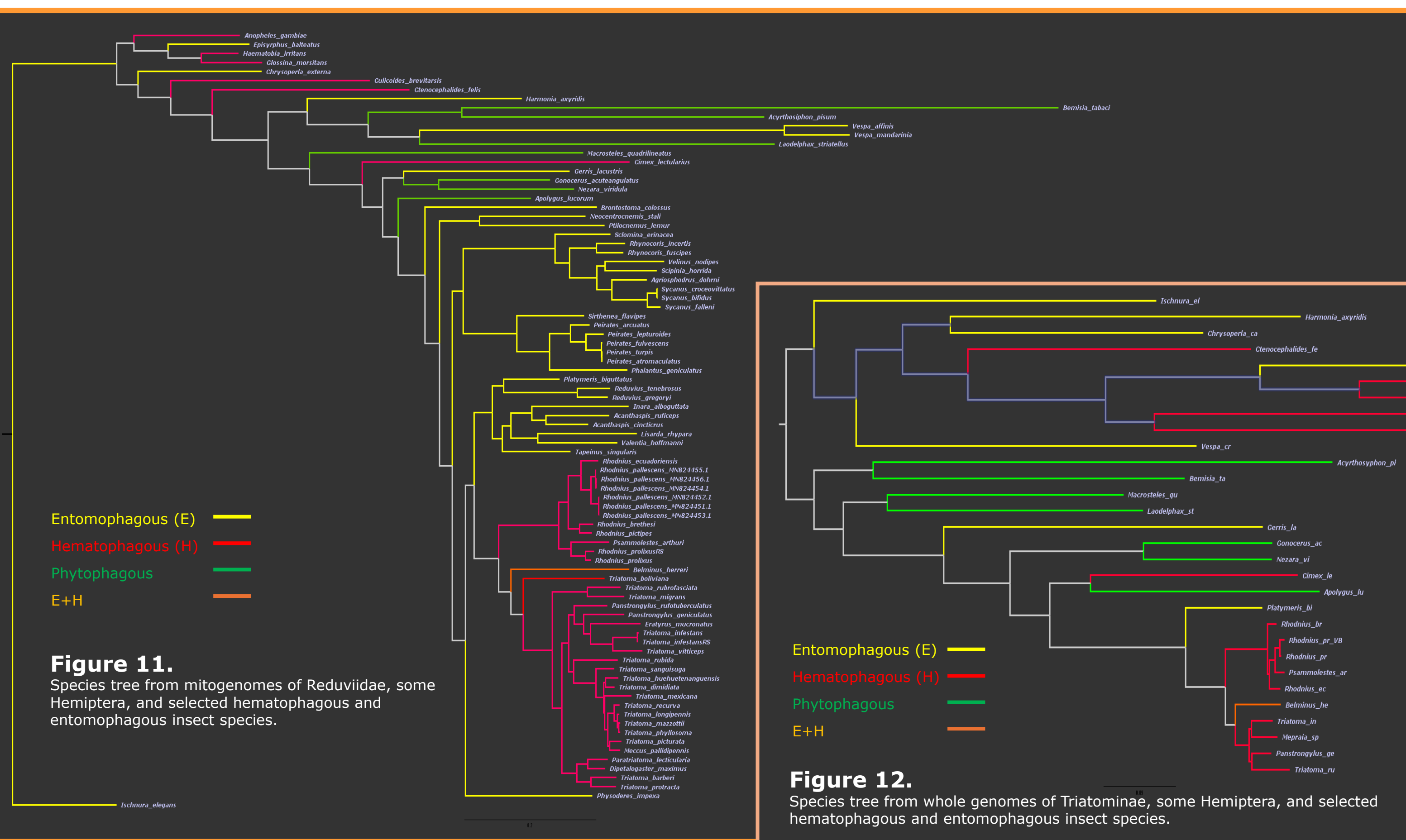


## Methods

We gathered samples from species across Latin America, extracted the DNA and performed long-read (ONT) and short-read (Illumina) sequencing, assembly and annotation [Fig. 1]. Assemblies were performed with Flye and polished with Racon, or Masurca; RagTag was used for scaffolding. The annotation pipeline included a homology-based annotation with Gemoma (Keilwagen et al. 2019), using available annotations for *Rhodnius prolixus* (Mesquita et al. 2015), *Triatoma rubrofasciata* (Liu et al. 2019), *Cimex lectularius* (Rosenfeld et al. 2016) and *Acyrtosiphon pisum* (Li et al. 2019). The Gemoma annotation was included as input to the GenSAS pipeline (Humann et al. 2019), along with available protein and RNA-seq data. Mitogenomes were generated using the short reads in NOVOPlasty (Dierckxsens et al. 2016) and annotated with MITOS2 (Donath et al. 2019) in Proksee (Grant et al. 2023), and the phylogeny of triatomine species within Hemiptera was obtained using OrthoFinder (Emms & Kelly 2015, 2019) [Fig. 1].

## Results and Discussion

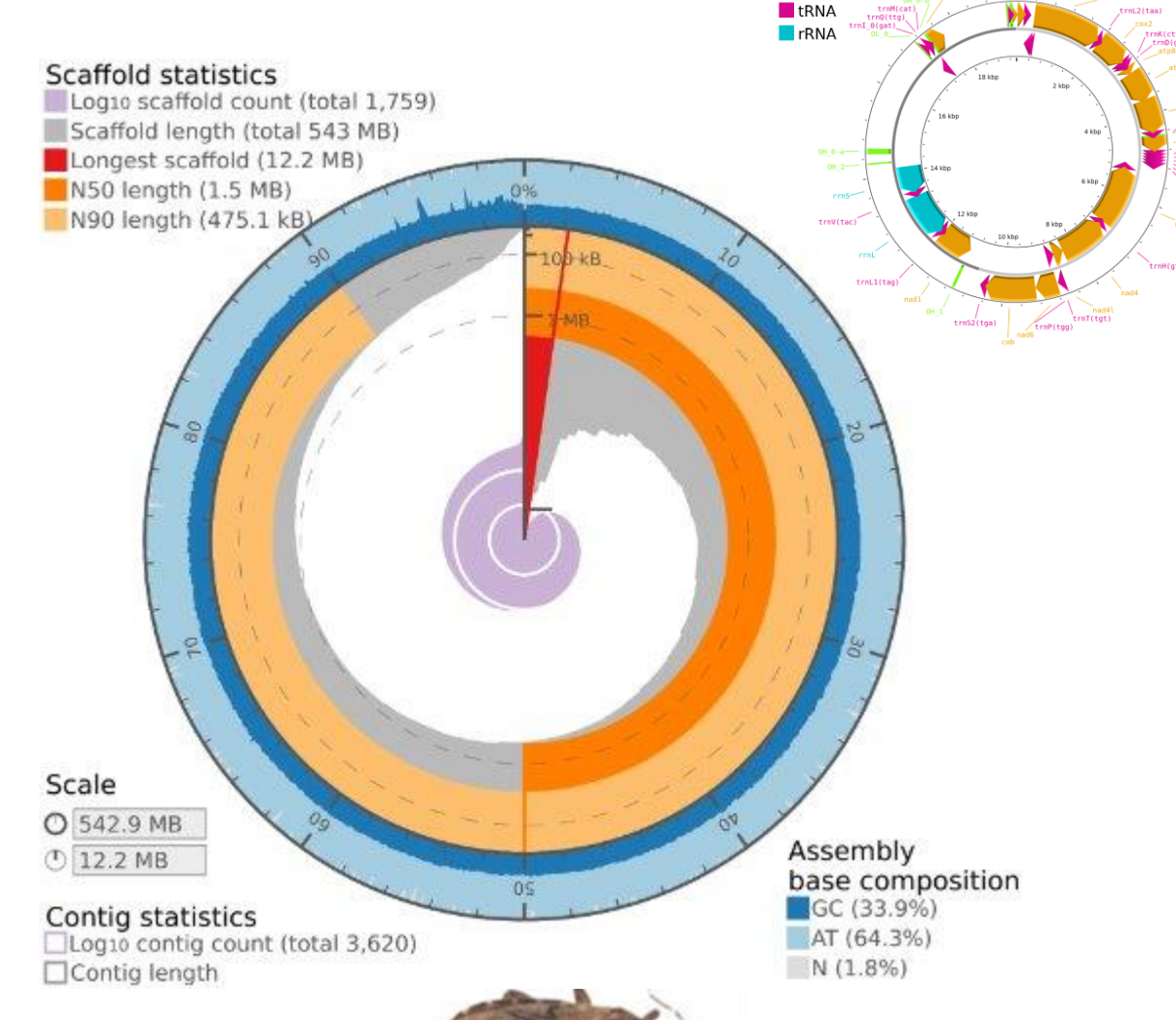
We produced eight new triatomine whole genome assemblies, for six species without previous genomes: *Belminus herrerii* (1.1 Gbp, GC 34.1%, N 0.9%) [Fig. 5], *Mepraia spinolai* (977.1 Mbp, GC 33.8%, N 0.6%) [Fig. 3], *Panstrongylus geniculatus* (1.2 Gbp, GC 34.7%, N 9.1%) [Fig. 4], *Psammolestes arthuri* (542.9 Mbp, GC 33.9%, N 1.8%) [Fig. 7], *Rhodnius brethesi* (550.7 Mbp, GC 33.5%, N 1.8%) [Fig. 8] and *Rhodnius ecuadoriensis* (583.8 Mbp, GC 33.9%, N 4.2%) [Fig. 9], and for two species with available but very fragmented assemblies: *R. prolixus* (583.9 Mbp, GC 34.0%, N 3.3%) [Fig. 10] and *Triatoma infestans* (1.1 Gbp, GC 33.8%, N 2.5%) [Fig. 2]. Furthermore, we also produced the first non-triatomine predatory reduviid whole genome assembly for *Platymeris biguttatus* (909 Mbp, GC 31.9%, N 0.8%) [Fig. 6], required for genomic comparisons. All of them present high gene completeness (BUSCOs >90%) [Figs. 14 & 15]. The mitogenomes show sizes over 15,900 bp, with mostly conserved gene order of the 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNAs and control region [Figs. 2, 4-10]. The species tree obtained with the mitogenomes [Fig. 11] and the whole genomes [Fig. 12] show similar results regarding Triatominae. Annotated genes related to hematophagy include lipocalins, triabins, odorant binding proteins, ionotropic receptors, and olfactory receptors, among many others. *Belminus herrerii* shows more similarity with hematophagous and entomophagous species than phytophagous ones, as expected [Fig. 13]. The preliminary results could indicate a polyphyletic origin of hematophagy in Triatominae, reopening the debate on this relevant aspect of Chagas disease vector biology, and stressing the need for increasing the genomic resources for this neglected illness.



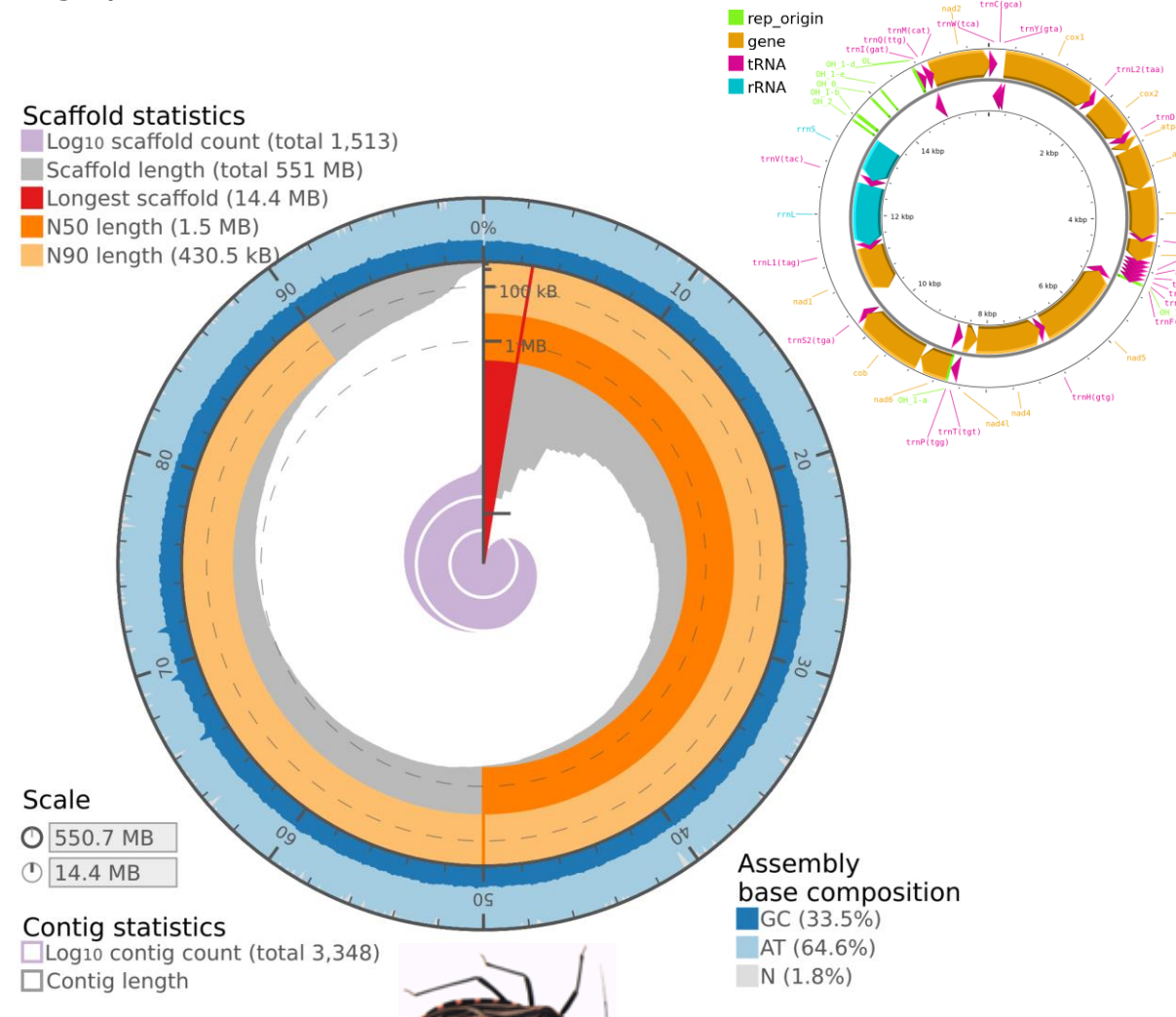
**Figure 5.** Tribe Bolboderini: *Belminus herrerii* whole genome assembly graphical representation. Mitogenome representation (top right).



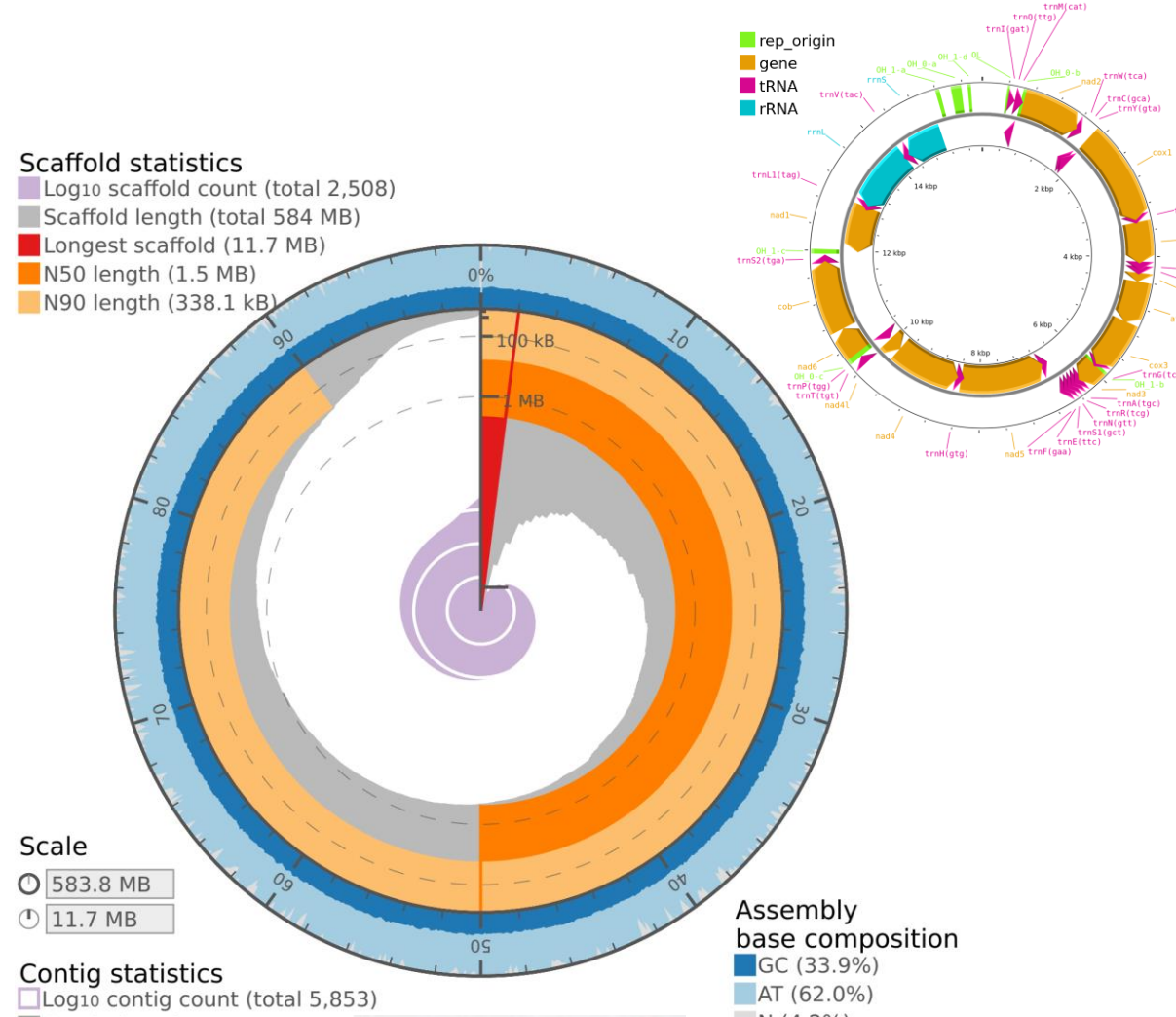
**Figure 6.** OUTGROUP: *Platymeris biguttatus* whole genome assembly graphical representation. Mitogenome representation (top right).



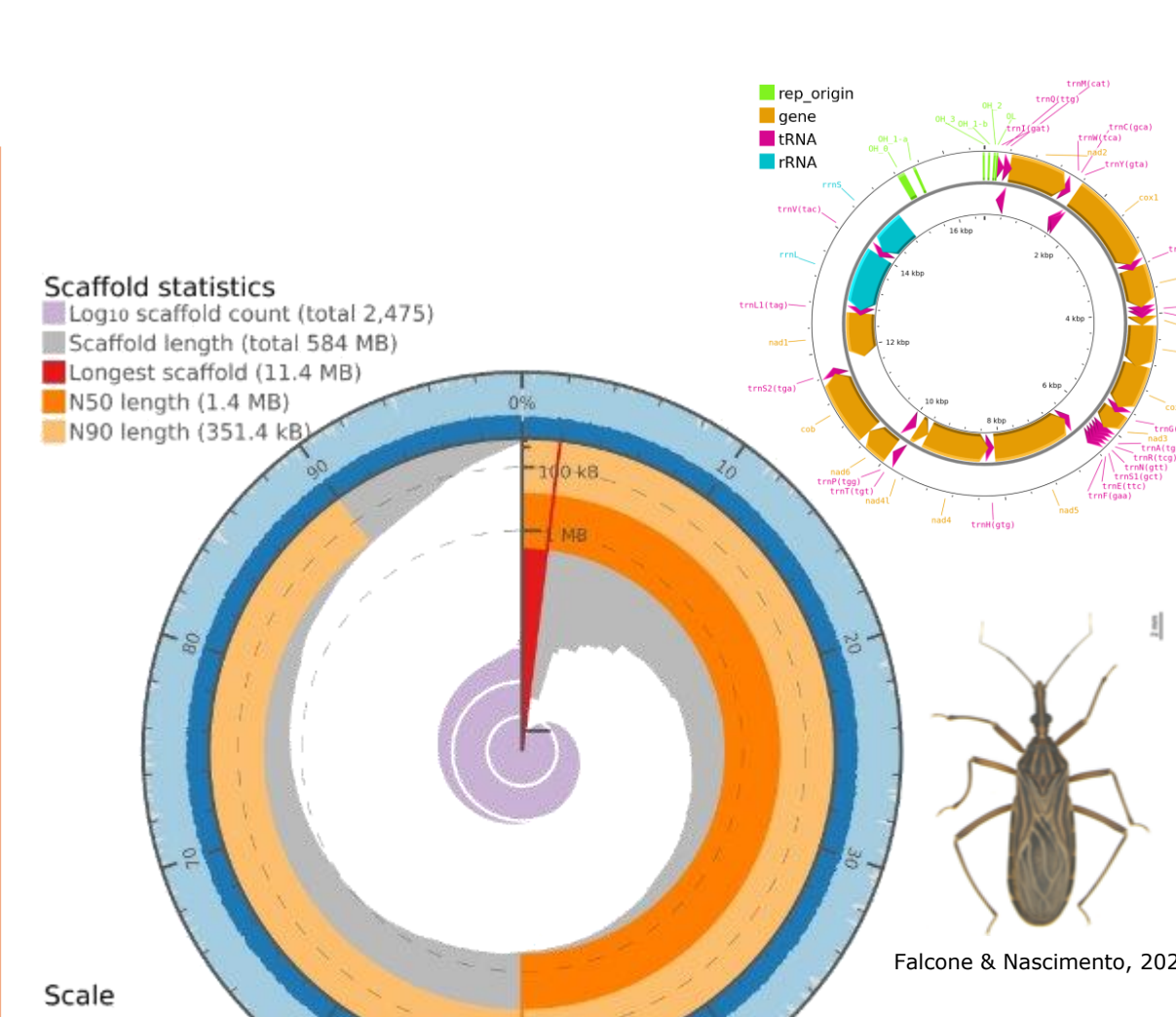
**Figure 7.** Rhodniini: *Psammolestes arthuri* whole genome assembly graphical representation. Mitogenome representation (top right).



**Figure 8.** Rhodniini: *Rhodnius brethesi* whole genome assembly graphical representation. Mitogenome representation (top right).



**Figure 9.** Rhodniini: *Rhodnius ecuadoriensis* whole genome assembly graphical representation. Mitogenome representation (top right).



**Figure 10.** Rhodniini: *Rhodnius prolixus* whole genome assembly graphical representation. Mitogenome representation (top right).

