# INTEGRATIVE TAXONOMY OF A NEW SPECIES OF THE GENUS *Pyrrhalta* Joannis, 1865 (Coleoptera: Chrysomelidae: Galerucinae) FROM SOUTHERN VIETNAM, WITH A KEY TO VIETNAMESE SPECIES

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#### **ABSTRACT**

A new species of genus *Pyrrhalta* (Chrysomelidae: Galerucinae), *Pyrrhalta dorsotibialis* n. sp., is described based on specimens from the Nui Chua National Park in southern Vietnam. Color photographs of habitus and body details, as well as an identification key for all Vietnamese species of the genus *Pyrrhalta* are provided. Moreover, *Menippus gressitti* Lee, Bezděk & Suenaga and *Pyrrhalta prokofievi* Skomorokhov are reported from Nui Chua, the latter as a remarkable finding, considering that the species was only known from the Con Dao Island. The availability of *cox1* DNA sequence data for these species in the Nui Chua Park allowed for a preliminary assessment of local phylogenetic diversity of this group compared with other homologous sequences publicly available for *Pyrrhalta* and the related genus *Menippus*. The two species currently recorded in the park seem to belong to at least two divergent phylogenetic lineages in the genus and highlighting the importance of the park for conservation of distant evolutionary branches of *Pyrrhalta*.

**Keywords**: Asia, biodiversity, COI, dry forest, taxonomy.

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### INTRODUCTION

The genus Pyrrhalta Joannis, 1865 (type species: Galeruca viburni Paykull, 1798) is widespread in the Palaearctic and Oriental regions, with one species also described from New Guinea (Medvedev, 2017). In addition, Pyrrhalta viburni, a native European species associated with Viburnum species, especially arrowwood viburnum, became adventive in northern North America (Majka & Lesage, 2007). Xue & Yang (2010) had listed up to 114 species of Pyrrhalta in the world. Years later, Nie et al. (2017), in their evaluation of the global diversity of subfamily Galerucinae, considered that the genus included 84 species as a conservative estimate. Meanwhile, several additional species have been described, including five from the Oriental region (Bezděk & Lee, 2019; Medvedev, 2019), one from New Guinea (Medvedev, 2017), one from Borneo (Takizawa, 2017), one from Sumatra (Bezděk & Lee, 2019), and nine from Taiwan (Bezděk & Lee, 2019; Lee & Bezděk, 2021). Moreover, two species of Pyrrhalta were transferred to Xanthogaleruca by Beenen & Talpur (2019). Thus, the current species count for the genus should be around one hundred species. However, the taxonomic limits of Pyrrhalta are still contentious and unclear.

In Vietnam, the first records of *Pyrrhalta* were reported by Samoderzhenkov (1988), who proposed nine species in the country, followed by seven additional species in Kimoto (1989), one species by Skomorokhov (2011), and two more by Medvedev (2012a, 2012b). A complete checklist and key to species of *Pyrrhalta* from Indochina were documented in Medvedev (2013), which together with new Vietnamese species described by Medvedev (2014), would represent a total of 22 species in Vietnam.

In this study, we report the species of *Pyrrhalta* and the related genus *Menippus* found in the Nui Chua National Park in southern Vietnam, including the description of a new species of *Pyrrhalta* and a key based on Medvedev's (2013) key of the genus, including all the species known from

Vietnam. We also use available molecular phylogenetic data for a preliminary assessment of the phylogenetic diversity of the genus in the park.

### **MATERIALS AND METHODS**

Specimens of Pyrrhalta were collected by beating the vegetation within arm's reach along transects in the Nui Chua National Park in Ninh Thuan province, southern Vietnam (Nguyen & Gómez-Zurita, 2016). analysis of morphology and photographs of specimens was done using a Leica M80 stereomicroscope with a Leica DF420 digital camera, or a Nikon Ds-Fi3 camera on a Nikon SMZ800N stereomicroscope and processed with NIS-element imaging software. Images of the same object taken with different focus planes were stacked using Helicon Focus 7 software. Species descriptions followed a similar treatment as in Nguyen & Gómez-Zurita (2017), and type specimens were deposited in the collection of Dinh Thi Nguyen (DTNC) at the Institute of Biology (IB), Ha Noi, Vietnam. The key of Indochinese Pyrrhalta by Medvedev (2013) was simplified to represent exclusively the species currently known from Vietnam, and the new species was integrated into the key. Nguyen & Gómez-Zurita (2016) produced DNA sequence data for the 3'-end of the cox1 mitochondrial DNA gene from the Pyrrhalta and Menippus specimens from the Nui Chua National Park, and they were available under accession numbers LT160479-LT160492 in the European Nucleotide Archive database (EMBL-EBI, Hinxton). These sequence data were analyzed in the context of other Pyrrhalta sequences available in public DNA sequence databases to investigate phylogenetic dispersion of the species present in the Nui Chua National Park. The phylogeny used representative sequences of all available Pyrrhalta species from public databases together with sequences of Menippus gressitti Lee, Bezděk & Suenaga (acc. no. LT160478) and Menippus dimidiaticornis Jacoby (acc. no. FJ977958). Sequences were aligned using the G-INS-I algorithm in MAFFT 7.3 (Katoh

& Standley, 2013). These data were analyzed with ModelTest-NG 0.1.7 (Darriba et al., 2020) to find the most suitable substitution model, and subsequently under maximum likelihood specifying this model in RAxML 8.2.12 (Stamatakis, 2014). The search for the best tree under maximum likelihood was based on 20 random starting topologies, and branch support was established based on 500 bootstrap pseudoreplicates. The same data were used to infer the phylogeny and branch probabilities under Inference with MrBayes 3.2.6 (Ronquist, 2001). Bayesian analysis consisted of four heated chains of 5M generations each, subsampling trees and parameters every 10K producing final estimates removing 10% of initial steps.

#### RESULTS

#### **Taxonomy**

Mennipus gressitti Lee, Bezděk & Suenaga, 2012

**Material examined: VIETNAM: Ninh Thuan prov.**: 1♂, Nui Chua N.P., Nui Ong mt., 11°43′19.8″N, 109°08′10.0″ E, 705 m, 3.V–6.V.2012, Dinh T. Nguyen leg. (DTNC).

This species is currently known from China (Hainan), Laos, Vietnam. In Vietnam, it was reported from the Central Highlands of Vietnam: Lam Dong province (mt. Langbiang & Dilinh), Dak Nong province (Dak Song), Dak Lak province (Ban Me Thuot). Our report from the Nui Chua National Park represents a remarkable expansion of the distributional range of the species, occupying different ecoregions.

# Pyrrhalta prokofievi Skomorokhov, 2011

Material examined: VIETNAM: Ninh Thuan prov.: 1♂, 3♀, Nui Chua N.P., Mainha mt., 11°43′11.7″N, 109°10′28.5″ E, 296 m, 26.I.2013–26.III.2013, Dinh T. Nguyen leg. (DTNC); 1♂, Nui Chua N.P., Mainha mt, 11°43′09.3″N, 109°11′28″, E 369 m, 20–30.VII.2012, Dinh T. Nguyen leg. (NTDC).

P. prokofievi was described recently from Con Dao National Park (Con Dao Island, South of Vietnam), and it was considered a local endemism of this isolated area. The presence of the species in the continent (Nui Chua National Park), in a region that is not too distant from the island, opens up the possibility that the island population was established from continental migrants.

*Pyrrhalta dorsotibialis* n. sp. (Figs. 1–3) urn:lsid:zoobank.org:pub:F3EEAF87-D92D-41B9-B722-216D928E49CA

**Type metarial**. Holotype ♂ (DTNC), VIETNAM: **Ninh Thuan prov**./. Nui Chua N.P./Ba Co mt., /11°42′0.78"N, 109°08′54.4" E/240 m/3.v–6.v.2012/ leg. Dinh T. Nguyen [w, p]//2891 [w, p]/". The specimen are provided with one additional printed label: "HOLOTYPUS ♂/Pyrrhalta dorsotibialis n. sp./Nguyen & Gómez-Zurita".

**Description**. Body entirely pale, except most of antennomere 4 and antennomeres 5–11, and dorsal surface of all tibiae black. Body length: 5.1 mm (Figs. 1, 2).

Head (Fig. 2a). Labrum transverse with round anterior border; disc with transverse row of several punctures with long, pale setae. Clypeus transverse, short, subtriangular with weakly concave and deflexed anterior border. Frontal ridge wide, short, sparsely covered with punctures and long setae; space between antennae wide, 1.85x transverse diameter of antennal socket. Eyes large, entire, slightly elongate dorsoventrally; frons wide, with interocular space 2.7x as large as transverse diameter of eye. Antennal calli slightly transverse, weakly raised, contiguous with thin median sulcus, separated from frons by weak depressions with row of short setae. Frons weakly convex, shiny, with thin longitudinal median suture, sparsely covered with small punctures and short setae.

Antennae filiform, 0.61x as long as body (Fig. 2b); pedicel shortest, antennomeres 4 and 5 longest; length proportions of antennomeres, 0.30: 0.20: 0.30: 0.32: 0.32: 0.29: 0.29: 0.27: 0.27: 0.25: 0.30.

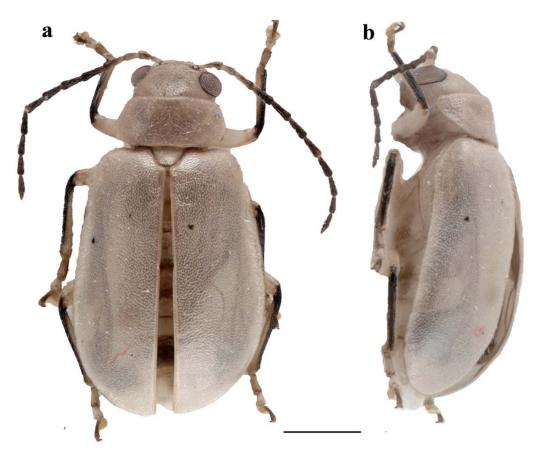


Figure 1. Dorsal (a) and lateral (b) views of the holotype of *Pyrrhalta dorsotibialis* n. sp. Scale bar: 1 mm for a, b

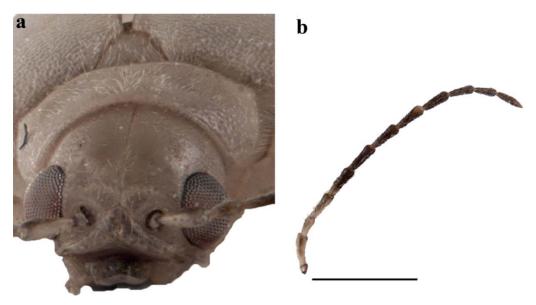


Figure 2. Head (a) and antenna (b) of the holotype of *Pyrrhalta dorsotibialis* n. sp. Scale bar: 1 mm for a, b



Figure 3. Penis of Pyrrhalta dorsotibialis n. sp. (a- dorsal view; b- abdomen view; c- lateral view; d- tip of penis). Scale bar: 1 mm for a–c

Pronotum transverse, 2.3x wider than long, widest in anterior third; sides moderately curved; disc smooth, with two shallow depressions at sides of disc, with heterogeneous punctures, larger in depressions and laterally,

sparsely with short pubescence. Scutellum large, subtriangular, with broad round apex, sparsely covered by small punctures and short setae. Elytra long, about 0.8x as long as body, broad, some 1.4x longer than wide combined, widened

at apical third; humeral calli strong; disc smooth, covered with small confused punctures, smaller than intervals, with short pubescence at base, sides and apex. Epipleura broad in basal quarter, gradually narrowing towards apex, densely covered with short setae. Legs without modifications; basitarsomeres weakly expanded laterally; second tarsomeres subtriangular, short; third tarsomeres shorter, transverse, weakly bilobed; length proportions of protarsomeres 0.30: 0.20: 0.16: 0.33; length of mesotarsomeres 0.34: 0.22: 0.16: 0.33; length of metatarsomeres 0.32: 0.23: 0.16: 0.33. Claws bifid.

Ventral surfaces covered with long setae. Penis 2.01 mm, broad in dorsal view, 3.8x longer than wide, sides subparallel, slightly curved laterally, with apex asymmetric, mucronate (Figs. 3a, b); weakly curved in lateral view, except near base (Fig. 3c); ostium large, with large asymmetric basal lamina; flagellum simple, blunt at apex, without teeth (Fig. 3d).

Female unknown.

**Differential** diagnosis. Pyrrhalta dorsotibialis n. sp. is similar to the species Pyrrhalta nigrotibialis, Pyrrhalta apicicornis having elytra fulvous without ridges or tubercules, pronotum entirely fulvous, underside fulvous, legs bicolor. The new species can be recognized from other Pyrrhalta species by the characteristic coloration of tibiae and antenna, and the shape of the penis: P. dorsotibialis n. sp with black dorsal surface of all tibiae, antennomeres 4-11 black; P. nigrotibialis with all tibiae and antennomeres 3-11 black; P. apicicornis with tarsi and most apices of tibiae black, antennomeres 4-7 black with fulvous bases.

**Distribution**. At present, the species is only known from the type locality.

**Etymology**. The specific name is derived from the noun *dorsum* (m.), referring to the upper side of a body or body part, and the adjective (f.) pertaining to the Latin noun *tibia*, or shinbone. The name relates to the obvious colour feature of tibiae in this species.

# A species identification key of the genus *Pyrrhalta* in Vietnam

1. Elytra with longitudinal or oblique ridges. Antennomere 10 not more than 1.5 times as long as wide
- Elytra without ridges or tubercles (sometimes with feeble elevation along anterior margin)
2. Elytron with one ridge
- Each elytron with 4 ridges. Upper surface reddish with spot on vertex, longitudinal stripe on prothorax, scutellum and sutural margin of elytra black; antennal underside and legs black. Body length 4.1–5.3 mm. Vietnam, Laos, Thailand, Myanmar
3. Elytron with one ridge originating from humerus and running parallel to lateral margin. Body entirely fulvous. Antennomere 10 about 3 times as long as wide. Penis as Fig. 11 in Medvedev (2013). Body length 5.5–7.1 mm. Vietnam, Laos, Thailand, China (Hainan)
- Elytron with a feeble ridge near side margin from anterior third to apical slope of elytron. Head, prothorax and scutellum fulvous, antennae entirely black, only antennomere 2 with fulvous base, elytra black with narrow lateral and more broad apical margins fulvous, underside dark fulvous, legs fulvous. Penis as Fig. 2 in Medvedev (2014). Body length 7.8 mm. Vietnam
4. Elytra mostly greenish
- Elytra without metallic green colour
5. Robust antennae, with preapical segments almost as long as wide6

- Antennae slender in preapical segments much wider than long. Fulvous, antennae blackish elytra greenish with marginal area fulvous. Body length 4.5 mm. Northern Vietnam (Originally described as <i>Chapalia jeanvoinei</i> Laboissière <i>P. jeanvoinei</i> (Laboissière, 1929)
6. Elytra greenish with lateral margin including epipleurae brownish. Body fulvous. Body length 9 mm. North Vietnam
- Generally greenish, with blue lateral margins on elytra and brownish on abdomen Anterior and posterior margins of prothorax not bordered. Body length 3.5–6.5 mm Northern Vietnam. (Originally described as <i>Decoomanius limbatus</i> Laboissière)
7. Upperside fulvous or reddish, sometimes with black pattern
- Elytra black. Head, pronotum and scutellum red. Antennae black with fulvous basa antennomeres, underside and femora fulvous, tibiae and tarsi black. Antennomere 10 about 3 times as long as wide. Pronotum with deep transverse impression, shallower in middle Penis as Fig. 12 in Medvedev (2013). Body length 4.1 mm. South Vietnam.
8. Elytra otherwise marked or entirely fulvous, pronotum not entirely black
9. Pronotum fulvous with black pattern or black spots10
- Pronotum entirely fulvous or reddish.
10. Pronotum with 3–4 black spots
- Pronotum with large transverse black spot in middle. Body fulvous, labrum except sides large spot on vertex, 2 elongate spots at base of elytra and apical half of elytra except margins black. Antennomere 10 about 3 times as long as wide. Penis as Fig. 14 in Medvedev (2013). Body length 8.2 mm. South Vietnam
11. Pronotum with three black spots.
- Pronotum with 4 black spots: 2 in front of base and 2 elongate near anterior angles. Vertex with 2 elongate black stripes; scutellum black. Body length 7.25 mm. Southern Vietnam (Con Dao Island and Nui Chua National Park)
12. Elytra without spots. Spot on the vertex, apices of the antennomeres, scutellum, apices of the tibiae and tarsi black. Antennomere 10 about twice as long as wide. Penis as Fig. 13 in Medvedev (2013). Body length 7.2–8.7 mm. South Vietnam
- Elytra with 5 black spots; middle of vertex, scutellum and metasternum black Antennomere 10 a little longer than wide. Body length 3.4–4.5 mm. South Vietnam Thailand, southeast China, Taiwan
13. Elytra fulvous or pale fulvous with black pattern14
- Upper surface, entirely fulvous or pale
14. Elytra not paler than pronotum
- Elytra pale flavous (much lighter than red fulvous head and prothorax) with margins narrowly black. Antennae black; underside fulvous with black metaventrite; legs fulvous with black upper surface of tibiae. Antennomere 10 about twice as long as wide. Penis as Fig. 15 in Medvedev (2013). Body length 4.5–4.6 mm. South Vietnam

15. Elytra with black basal band. Antennomere 10 about 3 times as long as wide. Underside fulvous
- Elytra differently marked
16. Basal band of elytra not reaching lateral margin, suture fulvous, apical slope without black spot. Legs fulvous with more or less blackish upper surface of tibiae. Penis as Fig. 16 in Medvedev (2013). Body length 5.1–7.2 mm. South Vietnam. Food recorded mainly <i>Tremo</i> (Ulmaceae), but also <i>Callicarpa</i> (Verbenaceae)
- Elytral basal band extending to lateral margin, suture narrowly black, apical slope with black spot. Legs fulvous with black tibiae and darkened tarsi. Body length 7.4 mm. Northern Vietnam
17. Elytra with black longitudinal stripe from humeral tubercle along lateral margin and transverse spot at base, sometimes connected with stripe, very rarely stripe is reduced. On elytra fulvous, metaventrite and abdomen sometimes more or less darkened. Antennomera 10 about 4 times as long as wide. Penis as Fig. 17 in Medvedev (2013). Body length 5.8–6.8 mm. South Vietnam
- Elytra? fulvous with elytral suture narrowly black; vertex, antennae except bases scutellum, meso- and metaventrite black; upper side of tibiae and tarsi more or less darkened. Antennomere 10 about twice as long as wide. Penis as Fig. 18 in Medvedev (2013). Body length 3.8–4.0 mm. North Vietnam
18. Legs bicolor. Underside fulvous
- Legs entirely fulvous.
19. All tarsi black
- Dorsal surface of tibia black, tarsi pale, except onychia and margins of basitarsomeres infuscate, antennomeres 4–11 black, dorsal surfaces of scape, and apical 2/3 of antennomere 3 dark. Penis as in Fig. 3. Body length 5.1 mm. South of Vietnam: Nui Chua National Park
20. Legs fulvous with black tibiae and tarsi. Antennae black, the 2 basal antennomeres fulvous. Antennomere 10 about twice as long as wide. Penis as Fig. 19 in Medvedev (2013) Body length 9.5–11.5 mm. South Vietnam
- Legs fulvous with tarsi and mostly apices of tibiae black. Antennae with antennomeres 1–3 and 8–11 fulvous, 4–7 black with fulvous bases. Antennomere 10 about 2.5 times as long as wide. Penis as Fig. 20 in Medvedev (2013). Body length 8.4–9.8 mm. Northern Vietnam.  ———————————————————————————————————
21. Antennae entirely fulvous
- Antennae partly black23
22. Pronotum with longitudinal impression in middle, widened before scutellum and deep round groove on each side. Antennomere 10 about 4 times as long as wide. Body? fulvous metaventrite and abdomen sometimes more or less darkened. Penis as Fig. 17 in Medvedev (2013). Body length 5.8–6.8 mm. South Vietnam <i>P. ornatipennis</i> Samoderzhenkov, 1988
- Pronotum without longitudinal impression in middle, with shallow round impressions or each side. Antennomere 10 about 3 times as long as wide. Body entirely fulvous. Penis as Fig. 21 in Medvedev (2013). Body length 6.8–8.7 mm. North Vietnam (Tam Dao), China
23 Rasal antennomeres fulvous

# Phylogenetic diversity of *Pyrrhalta* in the Nui Chua National Park

The Chrysomelidae fauna of Nui Chua National Park includes two species of Pyrrhalta at present, one of them newly described in this work and only known from its type locality in the Park, and a second one, P. prokofievi, previously known from Con Dao National Park, a small archipelago some 440 km SW from the Nui Chua Park, off the delta of the Mekong river (Skomorokhov, 2011). Moreover, we confirmed the presence in the park of a representative of the closely related specifically genus Menippus, M. gressitti, a species previously known from the Central Highlands of Vietnam (Kimoto, 1989; Lee et al., 2012).

The substitution model with best fit to the cox1-dataset of Menippus and Pyrrhalta TMV+G+I. sequences was a and implementation maximum likelihood to inference generated an optimal tree with a likelihood score = -3910.5934, lacking enough support and resolution to group confidently most of the species. The Bayesian tree for the same dataset analyzed under a GTR+G+I model (the best approach to TVM as allowed by MrBayes) showed a similar topology with similar lack of support for most relationships (Fig. 4). This preliminary phylogeny based on the gene tree of a fragment of the cox1 gene and including 14 species of Pyrrhalta, most of them from China, offers some insight into the phylogenetic diversity of this genus in Vietnam. Specifically, the two species of

Pyrrhalta in this work fall into two main clades of the Bayesian tree (the same clades appear in the maximum likelihood tree but without support). The new species appeared as a sister with moderate posterior probability (PP = 0.7) of the clade including the Chinese species Pyrrhalta griseovillosa (Jacoby) and Pyrrhalta sulcatipennis (Chen). In turn, P. prokofievi appeared as one of the isolated lineages in the clade including the remaining species of Pyrrhalta. The presence of at least two distantly diverged lineages of Pyrrhalta in the National Park suggests the presence of high environmental heterogeneity. This indicates the importance of conserving this area to preserve its unique ecological characteristics.

## **DISCUSSION**

The genus *Pyrrhalta* is one of the speciesrich genera of Galerucinae in the Oriental and southeastern Palaearctic Regions, together with the genus *Taumacera* Thunberg, 1814, which contains 97 species (Nguyen & Bezděk, 2021), or the genus Charaea Baly, 1878, which contains 60 species (Nguyen et al., 2021). The taxonomic limits of the genus Pyrrhalta are unclear, and taxonomic changes, including new synonymies of genera and new combinations of species, are frequently made. Pyrrhalta, Tricholochmaea Laboissière, 1932, and Xanthogaleruca Laboissière, 1934 had been treated as valid genera (Nie et al., 2017; Warchalowski, 2010), but Lee & Bezděk (2021) placed Tricholochmaea as part of Pyrrhalta, and Nie et al. (2012) treated Xanthogaleruca as a synonym of *Pyrrhalta. Pyrrhalta shaanxiana* Medvedev was synonymized with *Menippus beeneni* Lee, Bezděk et Suenag (Bezděk & Beenen, 2020). In these circumstances,

molecular phylogenies can show their full potential to address an objective delimitation of the genus and a robust internal classification.

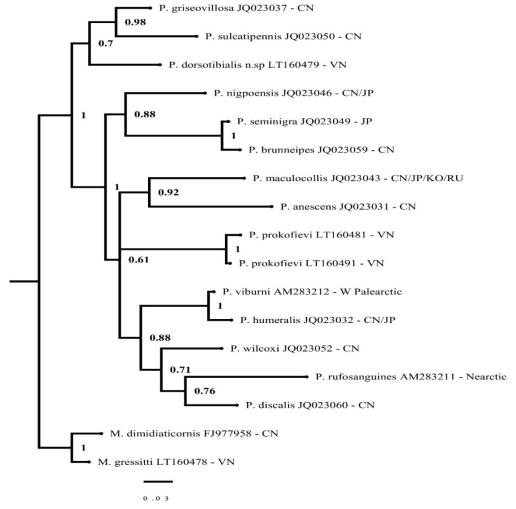


Figure 4. Bayesian inference tree on cox1 data of Pyrrhalta showing clade posterior probabilities > 0.60. The species are identified with their corresponding accession number in GenBank and information about their distribution (CN: China; JP: Japan; KO: Korea; RU: Russia; VN: Vietnam)

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