

# Rosaceae, Brassicaceae and Pollen Beetles: exploring relationships and evolution in an anthophilous beetle lineage (Nitidulidae, Meligethes-complex of genera) using an integrative approach

MEIKE LIU

Key Laboratory of Plant Protection Resources and Pest Management of Ministry of Education, Entomological Museum, Northwest A&F University, Yangling, Shaanxi; College of Agriculture, Yangtze University, Jinghou, Hubei

MIN HUANG

Key Laboratory of Plant Protection Resources and Pest Management of Ministry of Education, Entomological Museum, Northwest A&F University, Yangling, Shaanxi

ANDREW RICHARD CLINE

Plant Pest Diagnostic Centre, California Department of Food & Agriculture, Sacramento, CA

EMILIANO MANCINI

Università degli Studi di Roma La Sapienza Dipartimento di Biologia e Biotecnologie Charles Darwin

ANDREA SCARAMUZZI

Università degli Studi di Roma La Sapienza Dipartimento di Biologia e Biotecnologie Charles Darwin

SIMONE PARADISI

Università degli Studi di Roma La Sapienza Dipartimento di Biologia e Biotecnologie Charles Darwin

PAOLO AUDISIO

Università degli Studi di Roma La Sapienza Dipartimento di Biologia e Biotecnologie Charles Darwin

DAVIDE BADANO

Università degli Studi di Roma La Sapienza Dipartimento di Biologia e Biotecnologie Charles Darwin; Università di Genova, Dipartimento Scienze della Terra dell'Ambiente e della Vita

Simone Sabatelli (✉ [simone.sabatelli@uniroma1.it](mailto:simone.sabatelli@uniroma1.it))

Università degli Studi di Roma La Sapienza Dipartimento di Biologia e Biotecnologie Charles Darwin <https://orcid.org/0000-0002-8282-7882>

---

## Research

**Keywords:** Pollen beetles, Rosaceae, Brassicaceae, evolution, host-shift, Palaearctic Region

**Posted Date:** June 26th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-37852/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

---

**Version of Record:** A version of this preprint was published on March 6th, 2021. See the published version at <https://doi.org/10.1186/s12983-021-00390-4>.

## Abstract

**Background:** *Meligethes* are pollen-beetles associated with flowers of Rosaceae as larvae. This genus, in its present-day concept, consists of 63 known species in two subgenera, *Meligethes* and *Odonthogethes*, predominantly occurring in the eastern Palaearctic. We analyzed 61 morphological and ecological characters (128 states) of all species, as well as of 7 outgroup species from 7 Meligethinae genera (including the believed sister-genus *Brassicogethes*), to investigate their phylogeny. A parallel molecular analysis was carried out on 9 *Meligethes*, 9 *Odonthogethes*, 3 *Brassicogethes* and 2 *Meligethinus* species, based on DNA sequence data from mitochondrial (COI, 16S) and nuclear (CAD) genes, to obtain additional phylogenetic information on the group.

**Results:** Morphological phylogenetic reconstructions supported the monophyly of the genus, and clades corresponding to purported subgenera *Meligethes* and *Odonthogethes*. Main species-groups were mostly recovered intact, however some unresolved polytomies remained. Molecular data suggested a different scenario, placing members of *Brassicogethes* (including 42 mostly W Palearctic species associated with Brassicaceae) as sister to *Odonthogethes*, with this clade being sister to *Meligethes* s.str. This alternative phylogenetic assessment suggests that the monophyletic clades *Meligethes* s.str., *Odonthogethes* and *Brassicogethes* should be regarded alternatively as three subgenera of a monophyletic *Meligethes*, or three genera in a monophyletic genus-complex, with mutually monophyletic *Brassicogethes* and *Odonthogethes*. Molecular analyses estimated the origin of this lineage at ca. 14-15 Mya from a common stem including *Meligethinus*.

**Conclusions:** We hypothesize in the Middle Miocene (likely in Langhian Age), the first *Meligethes* specialized on Rosaceae, on which they subsequently radiated during Late Miocene and Plio-Pleistocene. This radiation was enforced by geographic isolation in E Asiatic mountain systems, and by larval host-plant specialization. Combined evidence from morphology, ancestral state parsimony reconstruction of host-plant associations, and molecular evidence, suggested that for *Meligethes* s.str., Rosoideae (*Rosa* spp.) represented the ancestral hosts, followed by an independent shift of ancestral *Odonthogethes* (ca. 9-15 Mya) on *Rubus* (Rosoideae) and members of Rosaceae Spiraeoideae. Other ancestral *Odonthogethes* probably shifted again on the unrelated plant family Brassicaceae (maybe 8-14 Mya in S China), allowing a rapid westward radiation of the *Brassicogethes* clade.

## Background

Nitidulidae, with almost 4500 known species, is a mid-sized family of the order Coleoptera. Within this taxon, the speciose subfamily Meligethinae comprises ca. 700 pollen-eating species described worldwide [1–4]. Recently, the classification of Meligethinae underwent several changes in light of both molecular and morphological evidence, restricting the concept of several included genera, particularly in the case of the previously polyphyletic genus *Meligethes* Stephens, 1830 [2, 5–6]. *Meligethes*, even as presently bounded [2] (Figs. 1A (a), (b)), is still a rather species-rich genus, consisting of more than 60 species predominantly occurring in the Eastern Palaearctic [7–10] (Fig. 1B-C; Table 1). This group includes species which are all associated with flowers of Rosaceae as larvae [2]. The majority of the species of this clade are oligophagous, although some appear to be strictly monophagous [1, 9–10]. Some locally common *Meligethes* (e.g. *M. atratus* (Oliver, 1790), *M. flavimanus* Stephens, 1830, *M. violaceus* Reitter, 1873) also represent economically significant potential pests, attacking blossoms of ornamental roses (*Rosa* spp.) and plum trees (*Prunus* spp.), in Europe, Japan, and elsewhere [1, 11–13]. The closely related and purported sister genus *Brassicogethes* Audisio & Cline, 2009 (Figs. 1A (c), D), comprises instead some forty, mostly Western Palaearctic species, all associated with Brassicaceae [2, 5, 14–23], and a few species [e.g. *B. aeneus* (F.) and *B. viridescens* (F.)] represent economically important pests, massively attacking blossoms of oilseed rapes, broccoli, cauliflowers, and others.

Table 1  
Summary of information on all 62 described species of *Meligethes* s.str. and *Odonthogethes*.

specific epithet	author(s) and year of description	subgenus	species group	host- plant	distribution	type(s)	notes
<i>argentithorax</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	W and SW China (Qinghai, Sichuan)	CAR-MZUR	
<i>atratus</i>	(Olivier, 1790)	<i>Meligethes</i>	<i>atratus</i>	<i>Rosa</i> spp.	W Palaearctic Region, N China, Russia	MHNP	
<i>aurantirugosus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>aurantirugosus</i>	<i>Rubus</i> sp.?	W Nepal	IZAS	female unknown
<i>aureolineatus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SW China (Sichuan)	NMPC	female genitalia unknown
<i>auricomus</i>	Rebmann, 1956	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> sp.?	SE China (Fujian, Jiangxi)	SMF	
<i>aurifer</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	Central China (Shaanxi, Shanxi)	NMPC	
<i>auripilis</i>	Reitter, 1889	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SW and NW China (Sichuan, Yunnan, Gansu, Shanxi, Shaanxi)	unknown	
<i>auropilosus</i>	Liu, Yang, Huang, Jelínek & Audisio, 2016	<i>Meligethes</i>	<i>nepalensis</i>	<i>Rosa</i> spp.?	SW and Central China (Xizang, Sichuan, Hubei, Shaanxi)	IZAS	
<i>aurorugosus</i>	Liu, Yang, Huang, Jelínek & Audisio, 2016	<i>Odonthogethes</i>	<i>aurantirugosus</i>	<i>Rubus</i> sp.?	W China (Xizang)	IZAS	female unknown
<i>binotatus</i>	Grouvelle, 1894	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	NE India, Nepal, SW China (Yunnan, Sichuan), N Myanmar, Bhutan	MHNP	
<i>bourdillonii</i>	Easton, 1968	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	E Nepal	BMNH	
<i>brassicogethoides</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	SW China (Yunnan)	NMPC	male unknown
<i>castanescens</i>	Grouvelle, 1903	<i>Odonthogethes</i>	<i>ferrugineus</i>	unknown	N India (Darjeeling), SW China (Yunnan)	MHNP	

specific epithet	author(s) and year of description	subgenus	species group	host- plant	distribution	type(s)	notes
<i>chinensis</i>	Kirejtshuk, 1979	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> spp.	W and Central China (Xizang, Yunnan, Sichuan, Chongqing, Gansu, Shaanxi, Henan, Hubei)	ZIN	
<i>cinereoargenteus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SW China (Sichuan)	NMPC	
<i>cinereus</i>	Jelínek, 1978	<i>Meligethes</i>	<i>nepalensis</i>	<i>Rosa</i> sp.?	Bhutan	NHMB	
<i>clinei</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> sp.?	SW China (Yunnan)	CAS	female unknown
<i>cyaneus</i>	Easton, 1957	<i>Meligethes</i>	<i>atratus</i>	<i>Rosa</i> sp.?	Japan	BMNH	
<i>denticulatus</i>	(Heer, 1841)	<i>Odonthogethes</i>	<i>denticulatus</i>	<i>Rubus</i> spp.	W Palaearctic Region, N China, Russia	ETHZ	
<i>elytralis</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SW China (Sichuan)	NMPC	
<i>ferrugineus</i>	Reitter, 1873	<i>Odonthogethes</i>	<i>ferrugineus</i>	unknown	N India (Sikkim)	MHNP	
<i>ferruginoides</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>ferrugineus</i>	<i>Pyracantha</i> sp.	Central and SW China (Hubei, Sichuan)	NMPC	
<i>flavicollis</i>	Reitter, 1873	<i>Odonthogethes</i>	<i>flavicollis</i>	<i>Photinia</i> sp. ?	E Russia, Japan, North Korea, SW, SE and Central China (Henan, Zhejiang, Chongqing, Jiangxi, Taiwan)	BMNH	
<i>flavimanus</i>	Stephens, 1830	<i>Meligethes</i>	<i>atratus</i>	<i>Rosa</i> spp.	W Palaearctic Region, N China, Russia	BMNH	
<i>griseus</i>	Jelínek, 1978	<i>Meligethes</i>	<i>nepalensis</i>	<i>Rosa</i> sp.?	Bhutan	NHMB	
<i>hammondi</i>	Kirejtshuk, 1980	<i>Meligethes</i>	<i>atratus</i>	<i>Rosa</i> spp.	W and central China (Shaanxi, Sichuan, Shanxi, Henan, Hubei)	BMNH	
<i>henan</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	Central China (Henan)	NMPC	

specific epithet	author(s) and year of description	subgenus	species group	host- plant	distribution	type(s)	notes
<i>inexpectatus</i>	Liu, Huang, Cline, Sabatelli & Audisio, 2017	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	SW China (Sichuan)	NWAU	male unknown
<i>lloydii</i>	Easton, 1968	<i>Odonthogethes</i>	<i>pectoralis</i>	<i>Malus</i> sp.?	Nepal, SW China (Yunnan)	BMNH	
<i>luteomaculatus</i>	Liu, Huang, Cline & Audisio, 2018	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	Central China (Hubei)	NWAU	
<i>luteoornatus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	SW China (Yunnan)	CAS	
<i>lutra</i>	Solsky, 1876	<i>Meligethes</i>	<i>vulpes</i>	<i>Rosa</i> spp.	Uzbekistan	ZMUM	
<i>macrofemoratus</i>	Liu, Yang, Huang, Jelínek & Audisio, 2016	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	Central China (Ningxia, Shaanxi, Hubei)	MHBU	
<i>marmota</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> sp.?	Nepal	MHNG	
<i>martes</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>vulpes</i>	<i>Rosa</i> sp.?	SW and N China (Shaanxi, Shanxi, Sichuan)	NMPC	
<i>melleus</i>	Grouvelle, 1908	<i>Meligethes</i>	<i>vulpes</i>	<i>Rosa</i> spp.	Myanmar, N India, N Pakistan, Afghanistan, S Tajikistan, Nepal	MNHN	
<i>nepalensis</i>	Easton, 1968	<i>Meligethes</i>	<i>nepalensis</i>	<i>Rosa</i> spp.	Nepal, N India	BMNH	
<i>nigroaeneus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	SW China (Yunnan)	CAS	
<i>nivalis</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> sp.?	SW China (Xizang, Yunnan, Chongqing)	NMPC	
<i>occultus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	SW China (Yunnan)	NMPC	male unknown
<i>pallidoelytrorum</i>	Chen & Kirejtshuk, 2013	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.	SW China (Sichuan)	IZAS	

specific epithet	author(s) and year of description	subgenus	species group	host- plant	distribution	type(s)	notes
<i>pectoralis</i>	Rebmann, 1956	<i>Odonthogethes</i>	<i>pectoralis</i>	<i>Malus</i> sp.	S Japan, SW, SE and Central China (Guizhou, Hubei, Fujian, Zhejiang, Taiwan)	SMF	
<i>pseudochinensis</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Pyracantha</i> sp.	Central China (Chongqing, Shaanxi, Hubei)	NMPC	
<i>pseudopectoralis</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>pectoralis</i>	<i>Malus</i> sp.?	SW China (Yunnan, Sichuan)	NMPC	
<i>sadanarii</i>	S.-T. Hisamatsu, 2009	<i>Odonthogethes</i>	<i>pectoralis</i>	<i>Malus</i> sp.?	SE China (Taiwan)	MNST	
<i>schuelkei</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	W China (Sichuan, Shaanxi?)	NMPC	
<i>scrobescens</i>	Chen, Lin, Huang & Yang, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	SW China (Sichuan, Hubei, Chongqing)	IZAS	
<i>semenovi</i>	Kirejtshuk, 1979	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SE Russia (Ussuri), Central, SW and NW China (Sichuan, Hubei, Shaanxi)	ZIN	
<i>shirakii</i>	S. Hisamatsu, 1956	<i>Odonthogethes</i>	<i>ferrugineus</i>	<i>Prunus</i> sp.?	S Japan (Kyū-Shū), SE China (Guizhou, Zhejiang, Taiwan)	EUMJ	
<i>simulator</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	Central China (Gansu, Shaanxi)	NMPC	
<i>stenotarsus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> sp.?	SW China (N Yunnan, Xizang)	NKMS	
<i>torquatus</i>	Jelínek, 1997	<i>Meligethes</i>	<i>atratus</i>	<i>Rosa</i> spp.	SE China (Taiwan)	NMPC	
<i>transmissus</i>	Kirejtshuk, 1988	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SW China (Sichuan, Yunnan)	ZIN	
<i>trapezithorax</i>	Liu, Huang, Cline & Audisio, 2018	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	Central China (Hubei)	NWAU	
<i>tricuspidatus</i>	Liu, Huang, Cline & Audisio, 2018	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	Central China (Hubei)	NWAU	male unknown

specific epithet	author(s) and year of description	subgenus	species group	host- plant	distribution	type(s)	notes
<i>tryznai</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SW China (Yunnan/ Xizang border)	NMPC	
<i>violaceus</i>	Reitter, 1873	<i>Meligethes</i>	<i>atratus</i>	<i>Rosa</i> spp.	China (Anhui, Shaanxi, Hubei, Zhejiang, Fujian, Guizhou, Jiangxi, Yunnan, Sichuan), SE Russia (Ussuri), Japan	BMNH	
<i>volkovichi</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>nepalensis</i>	<i>Rosa</i> sp.?	SW China (Yunnan)	CAS	
<i>vulpes</i>	Solsky, 1876	<i>Meligethes</i>	<i>vulpes</i>	<i>Rosa</i> spp.	Uzbekistan, Kyrgyzstan, Tajikistan, Turkmenistan, NW China (Xinjiang)	ZMUM	
<i>wagneri</i>	Rebmann, 1956	<i>Odonthogethes</i>	<i>denticulatus</i>	<i>Sorbaria</i> sp.?	SE and Central China (Fujian, Zhejiang, Taiwan, E Shaanxi)	SMF	
<i>xenogynus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>ferrugineus</i>	<i>Rubus</i> sp.?	SW and central China (Sichuan, Shaanxi)	NMPC	
<i>yak</i>	Liu, Yang, Huang, Jelínek & Audisio, 2016	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> sp.?	SW China (Sichuan)	IZAS	female unknown

Table 1  
Summary of the information on all 62 [*Meligethes* s.str. + *Odonthogethes*] described species.

specific epithet	author(s) and year of description	subgenus	species group	host- plant	distribution	type(s)	notes
<i>argentithorax</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	W and SW China (Qinghai, Sichuan)	CAR-MZUR	
<i>atratus</i>	(Olivier, 1790)	<i>Meligethes</i>	<i>atratus</i>	<i>Rosa</i> spp.	W Palearctic Region, N China, Russia	MHNP	
<i>aurantirugosus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>aurantirugosus</i>	<i>Rubus</i> sp.?	W Nepal	IZAS	female unknown
<i>aureolineatus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SW China (Sichuan)	NMPC	female genitalia unknown
<i>auricomus</i>	Rebmann, 1956	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> sp.?	SE China (Fujian, Jiangxi)	SMF	
<i>aurifer</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	Central China (Shaanxi, Shanxi)	NMPC	
<i>auripilis</i>	Reitter, 1889	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SW and NW China (Sichuan, Yunnan, Gansu, Shanxi, Shaanxi)	unknown	
<i>auropilosus</i>	Liu, Yang, Huang, Jelínek & Audisio, 2016	<i>Meligethes</i>	<i>nepalensis</i>	<i>Rosa</i> spp.?	SW and Central China (Xizang, Sichuan, Hubei, Shaanxi)	IZAS	
<i>aurorugosus</i>	Liu, Yang, Huang, Jelínek & Audisio, 2016	<i>Odonthogethes</i>	<i>aurantirugosus</i>	<i>Rubus</i> sp.?	W China (Xizang)	IZAS	female unknown
<i>binotatus</i>	Grouvelle, 1894	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	NE India, Nepal, SW China (Yunnan, Sichuan), N Myanmar, Bhutan	MHNP	
<i>bourdilloni</i>	Easton, 1968	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	E Nepal	BMNH	
<i>brassicogethoides</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	SW China (Yunnan)	NMPC	male unknown
<i>castanescens</i>	Grouvelle, 1903	<i>Odonthogethes</i>	<i>ferrugineus</i>	unknown	N India (Darjeeling), SW China (Yunnan)	MHNP	

specific epithet	author(s) and year of description	subgenus	species group	host- plant	distribution	type(s)	notes
<i>chinensis</i>	Kirejtshuk, 1979	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> spp.	W and Central China (Xizang, Yunnan, Sichuan, Chongqing, Gansu, Shaanxi, Henan, Hubei)	ZIN	
<i>cinereoargenteus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SW China (Sichuan)	NMPC	
<i>cinereus</i>	Jelínek, 1978	<i>Meligethes</i>	<i>nepalensis</i>	<i>Rosa</i> sp.?	Bhutan	NHMB	
<i>clinei</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> sp.?	SW China (Yunnan)	CAS	female unknown
<i>cyaneus</i>	Easton, 1957	<i>Meligethes</i>	<i>atratus</i>	<i>Rosa</i> sp.?	Japan	BMNH	
<i>denticulatus</i>	(Heer, 1841)	<i>Odonthogethes</i>	<i>denticulatus</i>	<i>Rubus</i> spp.	W Palearctic Region, N China, Russia	ETHZ	
<i>elytralis</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SW China (Sichuan)	NMPC	
<i>ferrugineus</i>	Reitter, 1873	<i>Odonthogethes</i>	<i>ferrugineus</i>	unknown	N India (Sikkim)	MHNP	
<i>ferruginoides</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>ferrugineus</i>	<i>Pyracantha</i> sp.	Central and SW China (Hubei, Sichuan)	NMPC	
<i>flavicollis</i>	Reitter, 1873	<i>Odonthogethes</i>	<i>flavicollis</i>	<i>Photinia</i> sp. ?	E Russia, Japan, North Korea, SW, SE and Central China (Henan, Zhejiang, Chongqing, Jiangxi, Taiwan)	BMNH	
<i>flavimanus</i>	Stephens, 1830	<i>Meligethes</i>	<i>atratus</i>	<i>Rosa</i> spp.	W Palearctic Region, N China, Russia	BMNH	
<i>griseus</i>	Jelínek, 1978	<i>Meligethes</i>	<i>nepalensis</i>	<i>Rosa</i> sp.?	Bhutan	NHMB	
<i>hammondi</i>	Kirejtshuk, 1980	<i>Meligethes</i>	<i>atratus</i>	<i>Rosa</i> spp.	W and central China (Shaanxi, Sichuan, Shanxi, Henan, Hubei)	BMNH	
<i>henan</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	Central China (Henan)	NMPC	

specific epithet	author(s) and year of description	subgenus	species group	host- plant	distribution	type(s)	notes
<i>inexpectatus</i>	Liu, Huang, Cline, Sabatelli & Audisio, 2017	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	SW China (Sichuan)	NWAU	male unknown
<i>lloydii</i>	Easton, 1968	<i>Odonthogethes</i>	<i>pectoralis</i>	<i>Malus</i> sp.?	Nepal, SW China (Yunnan)	BMNH	
<i>luteomaculatus</i>	Liu, Huang, Cline & Audisio, 2018	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	Central China (Hubei)	NWAU	
<i>luteoornatus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	SW China (Yunnan)	CAS	
<i>lutra</i>	Solsky, 1876	<i>Meligethes</i>	<i>vulpes</i>	<i>Rosa</i> spp.	Uzbekistan	ZMUM	
<i>macrofemoratus</i>	Liu, Yang, Huang, Jelínek & Audisio, 2016	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	Central China (Ningxia, Shaanxi, Hubei)	MHBU	
<i>marmota</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> sp.?	Nepal	MHNG	
<i>martes</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>vulpes</i>	<i>Rosa</i> sp.?	SW and N China (Shaanxi, Shanxi, Sichuan)	NMPC	
<i>melleus</i>	Grouvelle, 1908	<i>Meligethes</i>	<i>vulpes</i>	<i>Rosa</i> spp.	Myanmar, N India, N Pakistan, Afghanistan, S Tajikistan, Nepal	MNHN	
<i>nepalensis</i>	Easton, 1968	<i>Meligethes</i>	<i>nepalensis</i>	<i>Rosa</i> spp.	Nepal, N India	BMNH	
<i>nigroaeneus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	SW China (Yunnan)	CAS	
<i>nivalis</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> sp.?	SW China (Xizang, Yunnan, Chongqing)	NMPC	
<i>occultus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	SW China (Yunnan)	NMPC	male unknown
<i>pallidoelytrorum</i>	Chen & Kirejtshuk, 2013	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.	SW China (Sichuan)	IZAS	

specific epithet	author(s) and year of description	subgenus	species group	host- plant	distribution	type(s)	notes
<i>pectoralis</i>	Rebmann, 1956	<i>Odonthogethes</i>	<i>pectoralis</i>	<i>Malus</i> sp.	S Japan, SW, SE and Central China (Guizhou, Hubei, Fujian, Zhejiang, Taiwan)	SMF	
<i>pseudochinensis</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Pyracantha</i> sp.	Central China (Chongqing, Shaanxi, Hubei)	NMPC	
<i>pseudopectoralis</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>pectoralis</i>	<i>Malus</i> sp.?	SW China (Yunnan, Sichuan)	NMPC	
<i>sadanarii</i>	S.-T. Hisamatsu, 2009	<i>Odonthogethes</i>	<i>pectoralis</i>	<i>Malus</i> sp.?	SE China (Taiwan)	MNST	
<i>schuelkei</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	W China (Sichuan, Shaanxi?)	NMPC	
<i>scrobescens</i>	Chen, Lin, Huang & Yang, 2015	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	SW China (Sichuan, Hubei, Chongqing)	IZAS	
<i>semenovi</i>	Kirejtshuk, 1979	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SE Russia (Ussuri), Central, SW and NW China (Sichuan, Hubei, Shaanxi)	ZIN	
<i>shirakii</i>	S. Hisamatsu, 1956	<i>Odonthogethes</i>	<i>ferrugineus</i>	<i>Prunus</i> sp.?	S Japan (Kyū-Shū), SE China (Guizhou, Zhejiang, Taiwan)	EUMJ	
<i>simulator</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>Chinensis</i>	<i>Rubus</i> sp.?	Central China (Gansu, Shaanxi)	NMPC	
<i>stenotarsus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> sp.?	SW China (N Yunnan, Xizang)	NKMS	
<i>torquatus</i>	Jelínek, 1997	<i>Meligethes</i>	<i>atratus</i>	<i>Rosa</i> spp.	SE China (Taiwan)	NMPC	
<i>transmissus</i>	Kirejtshuk, 1988	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SW China (Sichuan, Yunnan)	ZIN	
<i>trapezithorax</i>	Liu, Huang, Cline & Audisio, 2018	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	Central China (Hubei)	NWAU	
<i>tricuspidatus</i>	Liu, Huang, Cline & Audisio, 2018	<i>Odonthogethes</i>	<i>chinensis</i>	<i>Rubus</i> sp.?	Central China (Hubei)	NWAU	male unknown

specific epithet	author(s) and year of description	subgenus	species group	host- plant	distribution	type(s)	notes
<i>tryznai</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> spp.	SW China (Yunnan/Xizang border)	NMPC	
<i>violaceus</i>	Reitter, 1873	<i>Meligethes</i>	<i>atratus</i>	<i>Rosa</i> spp.	China (Anhui, Shaanxi, Hubei, Zhejiang, Fujian, Guizhou, Jiangxi, Yunnan, Sichuan), SE Russia (Ussuri), Japan	BMNH	
<i>volkovichi</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Meligethes</i>	<i>nepalensis</i>	<i>Rosa</i> sp.?	SW China (Yunnan)	CAS	
<i>vulpes</i>	Solsky, 1876	<i>Meligethes</i>	<i>vulpes</i>	<i>Rosa</i> spp.	Uzbekistan, Kyrgyzstan, Tajikistan, Turkmenistan, NW China (Xinjiang)	ZMUM	
<i>wagneri</i>	Rebmann, 1956	<i>Odonthogethes</i>	<i>denticulatus</i>	<i>Sorbaria</i> sp.?	SE and Central China (Fujian, Zhejiang, Taiwan, E Shaanxi)	SMF	
<i>xenogynus</i>	Audisio, Sabatelli & Jelínek, 2015	<i>Odonthogethes</i>	<i>ferrugineus</i>	<i>Rubus</i> sp.?	SW and central China (Sichuan, Shaanxi)	NMPC	
<i>yak</i>	Liu, Yang, Huang, Jelínek & Audisio, 2016	<i>Meligethes</i>	<i>auripilis/ binotatus</i>	<i>Rosa</i> sp.?	SW China (Sichuan)	IZAS	female unknown

[Table 1 should appear here]—————

Most species of *Meligethes* and several within the related genus *Brassicogethes* have been recently analyzed by our research group through an integrated approach combining morphological, molecular, and bionomical data on larval ecology. This contribution deals with morphological and molecular data on adults, and on bionomical data on larvae. The thus far available molecular data set for *Meligethes* and *Brassicogethes*, including sequences of three mitochondrial and nuclear genes for 21 species (Table 2), allowed us to depict a first scenario of the phylogeny of *Meligethes* and of some related genera, to also provide a framework for understanding the origin of this group of Meligethinae, its evolution on different subfamilies of Rosaceae, and the shift of the first ancestral *Brassicogethes* to Brassicaceae.

Table 2  
should appear at (see page 3)

<i>Species</i>	<i>Sample ID</i>	<i>Localities</i>
<i>Meligethes auripilis</i>	MAU1_1	China-Sichuan, Kangding co.
<i>Meligethes auripilis</i>	MAU1_2	China-Sichuan, Kangding co.
<i>Meligethes auropilosus</i>	MAURO1_1	China-Hubei, Shennongjia Forest, Shennong Peak area
<i>Meligethes binotatus</i>	MBI1_1	China-Sichuan, Xiangcheng co.
<i>Meligethes chinensis</i>	4C2	China-Chongqing, Shizhu, Huangshui
<i>Meligethes elytralis</i>	MEL1_1	China-Sichuan, Xiangcheng co.
<i>Meligethes ferruginoides</i>	MFE1_1	China-Sichuan, Moxi, Yanzigou
<i>Meligethes hammondi</i>	MHA 1_1	China-Hubei, Shennongjia forest, Muyu
<i>Meligethes luteomaculatus</i>	MLU1_1	China-Hubei, Shennongjia Forest, Shennong Peak area
<i>Meligethes pallidoelytrorum</i>	MPA2_1	China-Sichuan, Ganzi-Moxi Town
<i>Meligethes pectoralis</i>	MPEC1_1	China-Guizhou, Tongzi-Louguan Mts, Wanmuhuahai
<i>Meligethes pectoralis</i>	MPEC1_2	China-Guizhou, Tongzi-Louguan Mts, Wanmuhuahai
<i>Meligethes pseudochinensis</i>	4A10	China-Hubei, Shennongjia forest, Muyu
<i>Meligethes pseudochinensis</i>	4C5	China-Chongqing, Shizhu, Huangshui
<i>Meligethes semenovi</i>	2C41	China-Tibet, Shannan, Cuona, Gongri
<i>Meligethes semenovi</i>	2C42	China-Tibet, Shannan, Cuona, Gongri
<i>Meligethes scrobescens</i>	4B7	China-Chongqing, Shizhu, Huangshui
<i>Meligethes transmissus</i>	MTR1_1	China-Sichuan, Kangding co.
<i>Meligethes violaceus</i>	MVI1_1	China-Zhejiang, Quzhou City, Jiangshan City
<i>Meligethes wagneri</i>	4C4	China-Chongqing, Shizhu, Huangshui
<i>Meligethes xenogynus</i>	3A31	China-Shaanxi, Meixian, Haoping temple
<i>Meligethes xenogynus</i>	3A32	China-Shaanxi, Meixian, Haoping temple
<i>Brassicogethes coracinus</i>	CR8_1	Turkey-Ardahan, road between Göle and Susuz
<i>Brassicogethes aeneus</i>	BAE13_3	Italy-Lazio, Pomezia, Borgo di Pratica di Mare
<i>Brassicogethes aeneus</i>	BAE13_4	Italy-Lazio, Pomezia, Borgo di Pratica di Mare
<i>Brassicogethes salvani</i>	BSA1.1	Italy-Piemonte, Mt. Palanfrè, Frisson lower Lake
<i>Meligethinus peringueyi</i>	MEP1_1	Mozambique-Maputo, Reserva Especial
<i>Meligethinus dolosus</i>	MED1_2	Mozambique-Maputo, Reserva Especial

[Table 2 should appear here]—————

Herein, we analyzed 56 morphological characters (with 128 character states) of adults (Fig. 5) for all 63 known *Meligethes* (s.l.) species (including a thus far undescribed species from S China), as well as 7 outgroup species belonging to 7 different related Meligethinae genera, including one representative species of *Brassicogethes*. Five additional bionomical characters were also analyzed combining available (published and unpublished) data on *Meligethes* larval ecology.

Although the genus *Meligethes* has been included in previous phylogenetic studies, those were aimed to resolve suprageneric classification using molecular approaches [24–26] and only few widespread W-Palaearctic species were represented. The present

work constitutes the first comprehensive phylogenetic analysis at the species level of this large group, with a focus on E Palaearctic lineages. Moreover, a preliminary interactive key to identification of the 62 described *Meligethes* species (based on MOSCH® software: Cerretti et al., 2012) is also ready to be available [at: [http://www.interactive-keys.eu/melikey/default\\_beta.aspx](http://www.interactive-keys.eu/melikey/default_beta.aspx)] [Liu et al., unpublished].

### The genus *Meligethes* Stephens, 1830

As recently summarized [7], the genus *Meligethes* (Figs. 1A (a), (b)) was formally established by Stephens in 1830 [27], based on the type species *Nitidula rufipes* Marsham, 1802 [present day valid name: *Meligethes atratus* (Olivier, 1790)]. A new preliminary phylogenetic scenario for *Meligethes* s.l., previously including a heterogeneous and obviously polyphyletic mixture of taxa, formally comprising as a whole more than 500 species worldwide, was presented recently [2, 24–26]. In the former paper [2], which was based on study of adult morphology and preliminary molecular data, 22 genera were described as new, and 6 previously recognized subgenera of *Meligethes* were elevated to generic rank, delimiting *Meligethes* to some thirty Palaearctic species that utilized Rosaceae as larval host-plants. Following the original descriptions of the few European species [28–30], several new or presumed new species were separately added from the Eastern Palaearctic and northern Oriental Regions by a long series of authors in the time span 1845/1997, including two important (although very preliminary) revisions [31–32] of both purported subgenera. In the most recent times two Chinese species were added [33–34], before the revision of the whole genus *Meligethes* [7], as recently delimited [2], where 23 new species (21 from China) have been described. Following this revision [7], a few other new species from China were also added [8–10], and other potential new species are under scrutiny based on recently collected Chinese material. The genus, comprising a peculiarly distinct new species still pending formal description, but considered in the present analysis (*M. sp. cfr. pectoralis* from S China; Tables 1, and 3–4 in additional files), now includes 63 species in two purported subgenera (*Meligethes* s.str. and *Odonthogethes* Reitter, 1871) [7–10] (Table 1).

Most *Meligethes* (s.l.) diversity occurs in the southern countries of the Eastern Palearctic and in the northern portions of the Oriental Region (Middle Asia, China, Japan, N Indian subcontinent); but China certainly represents the main hot-spot of the genus, ca. 85% of the known species being at least marginally distributed in this country (Figs. 1B–C, and Table 1) [7, 10].

Species belonging to this believed monophyletic genus are characterized by the following unequivocal autoapomorphic morphological and bionomical characters:

1) **temples behind eyes (posteriorlateral view)**: with distinct, deep elliptical pit, positioned inside the posterior terminal portion of the antennal grooves (Fig. 5a); or with distinct, shallow, subcircular pit, placed more dorsad, outside antennal grooves, close to the posterior-lateral edge of the eye (Fig. 5b; pits on temples or inside antennal grooves are absent in all other Meligethinae exhibiting not raised notosternal sutures). 2) **distal posterior portion of ventral antennal grooves (observed in ventral view)**: abruptly sloping, markedly delimited, deep, and distinctly wider than median portion (differently shaped in all other Meligethinae exhibiting not raised notosternal sutures). 3) **larval development**: on Rosaceae.

Several other morphological characters are shared with its purported sister genus *Brassicogethes* Audisio & Cline, 2009 (Fig. 1, A (c)) [2, 5, 24, 26], whose included species all develop as larvae on the unrelated plant family Brassicaceae. These characters include, but are not limited to: 1) terminal tarsomere simple, not toothed at base (in all *Meligethes* s.str.; slightly to strongly toothed at base in *Odonthogethes*) (Fig. 5k-m); 2) terminal maxillary palpomera long and slender, ca. 3 × longer than wide (Fig. 5e); 3) notosternal sutures usually not distinct, even in anterior portion (Fig. 5f), except in 3 species of *Meligethes* s.str.: *M. violaceus*, *M. torquatus* and *M. cyanus* (Tables 1 and 3–4 in additional files); 4) protibiae bearing only small, minute and subequal cuticular teeth along outer edge (Figs. 1A (a), (b), (c), 2 g); 5) protibiae usually long and slender, up to 4–4.5 × longer than wide (Figs. 1A (a), (b), (c), 2 g); 6) pronotum scarcely convex, at least partially flattened at sides, with posterior angles almost right or (in most *Meligethes* s.str.) slightly turned posteriad (Figs. 1A (a), (b), (c); 7) complete absence of circum-ocular furrows ("occipital sulci") when viewed dorsally (Figs. 2 (i), (j)); 8) semi-circular arched impressions on both sides of the proximal basal portion of the last abdominal ventrite large and markedly distinct (Fig. 2 (d)); 9) semi-circular arched impressions on both sides of the proximal basal portion of the pygidium arcuately and regularly convergent distad (Fig. 2 (n)); 10) male genitalia with plesiotypic tegminal shape, tegmen characterized in most species by a deep, V-shaped incision (Figs. 2 (o)–(r)), similarly exhibited by several other basal Meligethinae genera, e.g., *Meligethinus* Grouvelle, *Microptria* Grouvelle, *Pria* Stephens, *Microporum* Waterhouse, and *Cryptarchopria* Jelínek.

Among the 56 selected morphological characters, several traits were not included that are traditionally important in Meligethinae interspecific diagnostics: e.g., presence/absence of protruded and pointed pygidial apex in males and/or females; presence/absence of elytral rugosity in *Meligethes* s.str.; and certain traits of the male and female genitalia. Those traits, upon cladistics analysis, resulted manifestly plesiomorphic in different unrelated clades, and therefore were deemed scarcely informative or even confusing in a cladistics framework.

## The Rosaceae host-plants

Rosaceae is a middle-sized plant family that includes some 3000 species and a little less than 100 genera in 3 recognized subfamilies (Rosoideae, Spiraeoideae, and Dryadoideae) [35–36]. Although exhibiting a worldwide distribution, Rosaceae are particularly diverse in northern Hemisphere temperate forests, where several genera and species of woody shrubs and small trees are important components of local forest communities. The family is peculiar in producing several different and highly distinctive types of fruits, including economically important edible fruits such as apples, pears, peaches, apricots, prunes, strawberries, cherries, raspberries, and blackberries. *Meligethes* s.l. (i.e., *Meligethes* s.str. + *Odonthogethes*) specialize on several genera and species of Rosaceae, although, contrary to the majority of other Meligethinae lineages [2, 37–38], only large shrubs and small trees of the two main subfamilies Rosoideae and Spiraeoideae are utilized [10]. No *Meligethes* (nor other Meligethinae genera) are, in fact, known to develop as larvae on herbaceous Rosaceae (e.g. widespread and species-rich *Potentilla*, *Fragaria*, and *Geum*), despite species of these same plant genera being commonly used by adults of several different genera and species of Meligethinae as occasional food-plants [1].

Despite limited or incomplete information on the larval host-plants of some species, *Meligethes* s.l. appear to all be associated with Rosaceae species belonging to the genera *Rosa*, *Rubus*, *Malus*, *Prunus*, *Crataegus*, *Pyracantha*, *Sorbaria*, and *Photinia* [1, 7, 10]. A couple of these (*Rosa*, *Rubus*) belong to the subfamily Rosoideae [35], whereas others (*Malus*, *Prunus*, *Crataegus*, *Pyracantha*, *Sorbaria*, and *Photinia*) to the subfamily Spiraeoideae. The two most commonly used larval hosts of *Meligethes* s.l. species are *Rosa* L. and *Rubus* L. *Rosa*, representing the only known larval host of species in the subgenus *Meligethes* s.str., includes some 200 species worldwide; *Rubus*, representing the main larval hosts of species in the purported subgenus *Odonthogethes*, includes at least between 400 and 1000 species [35, 39–41]. Both genera exhibit biodiversity hot-spots in China and neighboring areas, where ca. 100 and more than 200 species respectively are known to occur [42]. A similar pattern occurs in *Meligethes* s.l., and in its constituent subgenera, species-groups and complexes [1–2, 7]. Due to these above mentioned botanical phylogenetic and biogeographic scenarios, we comprehensively explored the evolutionary trajectories within *Meligethes* s.l. to elucidate forms of ancient coevolutionary relationships between this group of pollen beetles and their larval hosts.

## Results

### Morphological phylogeny and cladistics analyses

The cladistic analysis of the matrix under implied weights yielded 567 equally most parsimonious trees with a total length of 160 steps, a consistency index (C.I.) of 0.42 and a retention index (R.I.) of 0.83 (Fig. 3).

*Brassicogethes* is recovered as the sister group to *Meligethes* based on a 1 nonhomoplasious apomorphy (37:0). The monophyly of *Meligethes* s.l. is in turn apparently supported by 3 nonhomoplasious (12:1; 53:1; 58:1) and 1 homoplasious apomorphies (48:1). The reciprocal relationships within *Meligethes* s.l. remained largely unclear, with 3 main clades emerging from a basal polytomy: A, B and C. The monophyly of clade A is based on 3 homoplasious apomorphies (26:1; 35:0; 44:0), although the relationships among the 4 members of this small clade (i.e., *M. lutra*, *M. martes*, *M. melleus* and *M. vulpes*) remained unresolved. Clade B was supported by 2 homoplasious apomorphies (20:0; 40:1) and includes 3 subclades, whose reciprocal relationships remained unclear and form a polytomy. The first subclade (which includes *M. auropilosus*, *M. nepalensis*, *M. volkovichi*, *M. cinereus* and *M. griseus*) was retrieved as monophyletic based on 1 nonhomoplasious apomorphy (9:1). The second subclade is supported by 1 nonhomoplasious (50:1) and 2 homoplasious apomorphies (35:0; 48:0). Within this group, *M. hammondi* is recovered as sister to a clade comprising *M. cyaneus*, *M. torquatus* and *M. violaceus*, which is in turn supported by 1 nonhomoplasious (25:1) and 1 homoplasious (2:1) apomorphies. The last subclade of B is established based on 1 nonhomoplasious apomorphy (24:1). Within the latter group, a clade comprising *M. cinereoargentatus* + (*M. aureolineatus* + *M. aurifer*) is based on 1 nonhomoplasious (8:1) and 1 homoplasious (20:1) apomorphies. This group is in turn sister to another clade based on 1 nonhomoplasious apomorphy (10:1), which in turn comprises two sister clades: of which the first ((*M. clinei* + *M. macrofemoratus*) + (*M. argentithorax* + *M. auripilis*)) is based on 1 nonhomoplasious apomorphy (6:1), while the second is recovered based on 1 nonhomoplasious apomorphy (4:1). Within the latter group, *M. marmota* is

sister to the rest of the clade, whose monophyly relies on a single homoplasious apomorphy (16:1). However, the internal relationships within this assemblage remained poorly resolved, with the exception of the well supported couplet comprising *M. nivalis* and *M. yak*, which is supported by 2 nonhomoplasious (17:1; 49:1) and 1 homoplasious apomorphies (46:0). The last of the main clades, C, which corresponds to the subgenus *Odonthogethes*, is well supported by 4 nonhomoplasious (13:1; 33:1; 44:1; 60:1) and 1 homoplasious apomorphies (27:0). Within clade C, the couplet + *M. schulkei* + *M. trapezithorax*, based on 1 homoplasious apomorphy (21:1), is found as sister to all the remaining members of the clade. Indeed, all remaining *Meligethes* species cluster based on 1 non homoplasious apomorphy (17:2), although the internal resolution of this group remains almost completely unresolved. *Meligethes denticulatus* and *M. wagneri* are sister based on 2 homoplasious (35:0; 43:1) apomorphies. One non homoplasious apomorphy (5:2) supports a clade comprising *M. luteomaculatus* + (*M. pallidoelytrorum* + *M. simulator*), while 1 homoplasious apomorphy (11:1) characterizes the clade including *M. nigroaeneus* + (*M. aurantirugosus* + *M. aurorugosus*). The remaining members of the genus group together in a clade supported by 1 nonhomoplasious (47:1) and 1 homoplasious (45:1) apomorphies, although their reciprocal relationships remained unresolved, forming a polytomy. However, a clade based on 1 homoplasious (59:1) apomorphy is derived from the assemblage. Within this group, *M. pseudochinensis* is sister to a clade based on 2 homoplasious (2:0; 35:0) apomorphies. This last clade comprises two sister groups, of which the first, including *M. shirakii* + (*M. xenogynus*, *M. castanescens* + (*M. ferrugineus* + *M. ferruginoides*)) is based on 1 nonhomoplasious (5:1) and 1 homoplasious (30:0) apomorphies. The remaining species cluster in a small clade based on 1 nonhomoplasious (1:1) apomorphy; within the latter clade *M. sp. cf. pectoralis* is sister to *M. flavigollis* + (*M. pectoralis* + (*M. lloydii*, *M. pseudopectoralis*, *M. sadanari*)).

#### Molecular phylogeny and divergence time estimation

Our final dataset consisted of 1841 bp (COI: 578 bp, 16S: 491 bp, CAD: 772) obtained from 28 specimens (see Table 2) and constituted the input for the BI and ML analyses. Phylogenetic trees resulting from the BI and ML analyses showed congruent topologies (Fig. 4); only BI posterior probability values and ML bootstrap values exceeding 70% are shown as BI/ML. Our phylogram indicate the presence of two highly supported principal clades, corresponding to the *Meligethes* s.str. species (BI = 1/ML = 100) and a clade which includes the *Odonthogethes* species and species belonging to genus *Brassicogethes* (BI = 1/ML = 92). Moreover, the monophyly and the sister-group relationships of a clade including *Meligethes* s.str. + [*Odonthogethes* + *Brassicogethes*] is well supported in both BI (0.85) and ML (81). The trees obtained from single-gene alignments are reported in additional files.

Divergence time estimates from BEAST are depicted in Fig. 5. With a calibration of 0.0126 substitutions/site per My, the possible origin of the core members of the *Meligethes* complex of genera [*Meligethes* s.str. + *Odonthogethes* + *Brassicogethes*] from *Meligethinus* is estimated to be approximately in the Middle Miocene, ca. 14–15 Mya. The split of *Odonthogethes* from *Meligethes* can be traced back to the Middle Miocene (12.27 Mya; 95% HPD: 9.10–15.30 Mya) and that of *Brassicogethes* from *Odonthogethes* in the Late Miocene (11.15 Mya; 95% HPD: 8.20–14.10 Mya).

#### Molecular vs. morphological analyses

Evidence from our molecular analyses resulted in rather good agreement with previously established morphology-based systematics of *Meligethes* and allied genera. Our molecular analyses suggest (Figs. 4–5) that most *Odonthogethes* species likely differentiated within the last 10 million years, mostly those included in the *O. chinensis* species-group, the largest species-group of the genus, which are associated as larvae with *Rubus* spp. (Rosoideae), and with several other Spiraeoideae. Although the taxon sampling in our molecular analysis is incomplete, preliminary conclusions and comparisons with the results of the morphological cladistic analysis, suggest that reconstructions agree in confirming the monophyly of the present-day *Odonthogethes*. Nevertheless, the monophyly of the (sub)genus *Meligethes* s. str. remains incompletely confirmed morphologically, suggesting that this taxon might be an assemblage from which *Odonthogethes* is derived. Future work with fresh material of representatives of clade A (Fig. 3; *Meligethes vulpes* and allied species, mostly from Middle Asia) is needed to verify, via molecular data, the phylogenetic position of this isolated group, whose unresolved morphologically based placement inside *Meligethes* s. str. determined the partially unresolved monophyly of this subgenus [or genus]. Additionally, our molecular data clearly and univocally suggest that members of the believed genus *Brassicogethes* originated from a stem of *Odonthogethes*, probably from a basal, ancestral species, which never developed one of the most striking morphological characters of this group, i.e., the toothed tarsal claws. This evidence is partially in contrast with morphological analyses, which, on the contrary, suggest a sister-group relationship of *Brassicogethes* with the clade [*Meligethes* s.str. + *Odonthogethes*]. In presence of this contrasting information, we believe that this not completely unexpected molecular evidence could be likely more reliable in reconstructing the true phylogenetic relationships among these taxa, therefore suggesting the need to downgrade *Brassicogethes* to the subgenus level, inside the genus *Meligethes* s. l., or, alternatively, to raise *Odonthogethes* to the genus rank. However, we decided to introduce and formalize this taxonomic proposal in a separate paper, specifically devoted to discuss more in detail the systematic consequences of the molecular evidence summarized herein.

## Ancestral state parsimony reconstruction of larval-host-plant associations

The ancestral state reconstruction plotted on the topology resulting from the combined morphological and molecular dataset analysis (Fig. 6) suggests that Rosaceae could be among the larval host plants of an early ancestor of the genera *Meligethes* (s. l.) and *Brassicogethes*, although the actual succession of family-level larval shifts among the whole clade is difficult to define.

Noteworthy, the members of the related genus *Meligethinus* are all associated with Monocots in the family Arecaceae [1–2, 46]. According to molecular data, the common ancestor of *Meligethes* s. l. subsequently colonized a new niche, probably exploiting Rosaceae in the Eastern Palaearctic, while *Brassicogethes*, maybe also originated in the same areas, later radiated on Brassicaceae, mostly in W Palaearctic areas. This scenario is supported by the ancestral state reconstruction, which suggested that the diverse genus *Rosa* L., represents the ancestral host of *Meligethes* s. str. with high likelihood. The larvae of most species of *Meligethes* s. str. specialized on species belonging to this plant group, while members of the purported subgenus *Odonthogethes* instead shifted on other Rosoideae, specifically on the genus *Rubus* L. Subsequently, a species of this subgenus colonized Rosaceae belonging to the subfamily Spiraeoideae and this ancestral host shift event allowed the diversification of a group whose members radiated by exploiting several plants of this subfamily (e.g. *Malus*, *Crataegus*, *Pyracantha* and *Photinia*) (Fig. 6). A few isolated species belonging to both subgenera, *Meligethes* (*M. atratus*, *M. flavimanus*, *M. torquatus*) and *Odonthogethes* (*O. shirakii*) independently colonized also Spiraeoideae in the genus *Prunus* [1, 13]. An even more isolated host shift event is represented by *Odonthogethes wagneri*, the only species feeding on Spiraeoideae of the genus *Sorbaria*, and the isolated *O. flavicollis*, which is the only species apparently feeding on Spiraeoideae of the genus *Photinia* [10]. Molecular data suggest that a more drastic ecological shift finally involved some ancestral species of *Odonthogethes*, able to transfer on Brassicaceae, and very likely originating the *Brassicogethes* clade.

## Discussion

*Meligethes* originated in the SE Palaearctic and NE Asia, and is linked to an adaptation to flowers and large inflorescences of Rosaceae. The genus is derived from an ancient stem of Meligethinae that is not distantly related from *Meligethinus*, *Micropria*, *Kabakovia*, *Pria*, and allied genera, several species of which (except among *Pria* and *Micropria*) are now associated as larvae with male inflorescences of palms (Arecaceae) [1–2, 24, 26, 46].

Inferences from available molecular data suggest that timing of separation between [*Meligethes* + [*Odonthogethes* + *Brassicogethes*]] occurred in a time frame of ca. 11–13 Mya, whereas separation of this clade from the related genus *Meligethinus* probably dates back to 14–15 Mya. Therefore, the Middle Miocene (Langhian Age), is best possible timing for the first *Meligethes* lineages to specialize on Rosaceae host plants. *Meligethes* later continued to evolve in Eastern Palaearctic and E Asiatic areas on Rosaceae Rosoideae, from which members of *Odonthogethes* radiated during the Late Miocene, Pliocene and Pleistocene on members of the Rosaceae subfamily Spiraeoideae. *Brassicogethes*, following their shift to Brassicaceae very likely from a stem of ancestral *Odonthogethes*, expanded and strongly diversified its constituent lineages mostly in W Palaearctic, paralleling a westward evolution, diversification and expansive radiation of Brassicaceae host-plants. Several closely related species of this (sub)genus likely differentiated only in the last 2 Mys [14, 22–23]. These species, in fact, usually exhibit low levels of molecular interspecific differentiation, typical of very recent speciation where genetic differentiation can be markedly slower than morphological and ecological differentiation. This phenomenon, well-known in different groups of recently speciated phytophagous insects, is probably due to widespread interspecific conservatism of common ancestral haplotypes in conditions where genetic differentiation has not had the necessary time to stabilize after speciation [23]. Among *Odonthogethes*, a similar situation certainly involves most members of the *Odonthogethes chinensis* group, which, although strongly differentiated from one another based on morphological and ecological traits, exhibit low levels of interspecific genetic differentiation (Fig. 5) [Audisio et al. unpublished data].

Only a few *Meligethes* of the (sub)genera *Meligethes* and *Odonthogethes* were able to reach the W Palaearctic, likely during the most recent Pleistocene Glaciation Cycles, maybe due to the moderate number of potential larval host-plants (indigenous Rosaceae) occurring west of Middle Asia. Mountain systems of the SE Palaearctic (in particular those in Central and S China, Nepal, Bhutan, and NE India) and transitional areas between E Palaearctic and N Oriental Regions, represent the most active centers of speciation and evolution of *Meligethes* and *Odonthogethes*, reflecting the local strong generic- and species-level diversification on Rosaceae [35–36, 40–42].

As mentioned above, *Meligethes* s. str. appear to be more strictly specialized on *Rosa* spp., while *Odonthogethes* appear to have adapted to a much wider range of Rosaceae genera, including: *Rubus* to *Prunus*, *Sorbaria* and related taxa, in the two main Rosaceae subfamilies, Rosoideae and Spiraeoideae [35–36, 47]. The only known more highly polyphagous *Meligethes* s.str. are represented by

two species (*M. atratus* and *M. flavimanus*) that are widespread from N Asia to the Iberian Peninsula. Both species are able to develop, at least in W Palaearctic, on different genera of Rosoideae and Spiraeoideae [1, 7]. This could be interpreted as a recent local widening of ecological (host-plants) ranges, following post-glacial colonization of W Europe, in a region where other competing and more specialized Rosaceae-dependent species of the same genus are absent.

However, our datasets showing that different phylogenetic information are potentially provided by morphological data and different molecular markers (see additional files). We are therefore confident that further molecular and morphological analyses could provide more detailed phylogenetic evidence on the three involved lineages, in a more shared scenario of their actual evolutionary relationships. Additionally, molecular data suggests a markedly isolated phylogenetic position for *Meligethinus dolosus* Grouvelle, 1919 (from NE South Africa and Mozambique). This evidence is confirmed by morphological data [46]. This problem, as well as the internal phylogeny of *Meligethinus*, is outside the scope of the present paper, and will be discussed in an upcoming effort that is specifically devoted to a morphological and molecular phylogeny of this genus [46].

## Conclusions

As reported in our *Meligethes* revision [7], and in more recent papers [8–10], 63 *Meligethes* (s.l.) species were analyzed in the present study and represents an additional work for further research on this species-rich taxon. Judging from the relatively high number of new species (> 12, PA pers. obs.) discovered during less than five years of field and museum research following the above cited revision, combined with the vast extension of scarcely explored E Palaearctic and N Asian areas (eastern Middle Asia, Northern Indian subcontinent, S China, and northern Indochina), there is a high likelihood that the actual number of species in the (sub)genera *Meligethes* and *Odonthogethes* might increase significantly to more than 70–80 species as a whole. The species of both clades seem to be concentrated in central and southern China, specifically in subtropical evergreen broadleaf forest zones, in eastern portions of the Qinghai-Xizang Plateau alpine vegetation zone, and in southern portions of the warm temperate deciduous-broadleaf forest zone [48] (<http://www.chinamaps.org/>

china/china-land-cover-map-large-2.html). However, scarcely explored mountain areas of S China still possess new endemic species (Liu et al. unpublished data). These areas represent true biodiversity hot-spots (Figs. 1B–C) for both *Meligethes* and *Odonthogethes* and their Rosaceae host-plants, and also likely include Nepal, Bhutan, Taiwan, and the nearly unexplored surrounding mountain areas of eastern India (Arunachal Pradesh) and northern Myanmar.

Despite the incomplete taxonomic and biogeographic coverage, the present morphological phylogenetic analysis and the thus far available molecular data seem to support at least the following conclusions:

- 1) Based on molecular evidence, the *Meligethes* complex of genera univocally represents a monophyletic lineage, including almost certainly three species-rich genera or subgenera (*Meligethes* s.str., *Odonthogethes*, and *Brassicogethes*), which are comparable in the number of inclusive species (32, 31 and 42 species, respectively). Other preliminary molecular data using mitochondrial and nuclear markers (COI, NADH, ITS2, PEPCK) [24, 26], suggested that the genus *Meligethinus* Grouvelle (a small, mostly Oriental and Afrotropical genus including some 20 species associated as larvae with male inflorescences of Arecaceae [1, 46]) is placed as sister of this clade. In fact, the phylogenetic position of *Meligethes* and *Odonthogethes* is certainly closer to both *Brassicogethes* and *Meligethinus*, than to any other recognized genera inside Meligethinae [46]. Indeed, the positional homologous insert at the apical portion of the ITS2 domain B, which, as recently demonstrated [24], is shared across all *Meligethes*, *Odonthogethes*, *Brassicogethes* and *Meligethinus*, should be considered a diagnostic sequence insertion. This combined with other slippage-derived signature sequences identified in the same paper for these related Meligethinae genera strongly corroborates a common origin of these four taxa.
- 2) Based only on morphological evidence (Fig. 3), the clade [*Meligethes* s.str. + *Odonthogethes*] could represent the sister-group of the genus *Brassicogethes*, with *Meligethinus* sister of this triplet of taxa.
- 3) The molecular data presented here (Figs. 4–5) strongly suggest a different and likely more compelling scenario, with *Brassicogethes* and *Odonthogethes* being sister taxa, *Meligethes* being sister of the latter clade, and *Meligethinus* sister of this triplet of taxa.
- 4) Finally, in an ecological-evolutionary perspective, there is evidence that *Meligethes* s. str. and *Odonthogethes* only rarely speciated in a pattern of strict coevolution with their larval host-plants. Most strictly endemic and locally distributed *Meligethes* and *Odonthogethes* species from China and neighboring countries are, in fact, typically associated as larvae with common and widespread floral hosts,

and not to strictly endemic plant species [10]. Therefore, geographic isolation and repeated host-shifts in more or less isolated mountain valleys seems to have played a more relevant role than ecological specialization and coevolution in *Meligethes* and *Odonthogethes* evolutionary history. On the contrary, in the mostly W Palaearctic *Brassicogethes* (associated with the unrelated family Brassicaceae) it is evident that several recently differentiated species evolved in connection with rare and endemic plant species [1, 5, 14–23]. Molecular evidence also suggests that the two largest genera of Rosaceae Rosoideae, *Rubus* and *Rosa*, split more than 40 Mya [40], well before the estimated divergence between *Odonthogethes* (mostly associated with *Rubus* spp.) and *Meligethes* s. str. (all likely associated with *Rosa* spp.) that is tentatively dated here (ca. 12–13 Mya). This combined dating strongly supports the lack of a direct coevolution between these beetles and host plants; perhaps with the exception of some recently diversified clades of plant genera and beetle species-complexes (e.g., members of the *Odonthogethes chinensis* complex on *Rubus* spp. in SW China).

6) An upcoming paper of our research team is aimed to introduce and formalize an updated taxonomic rank for *Meligethes*, *Odonthogethes*, and *Brassicogethes*, to describe a short series of very recently discovered new Chinese species, and to describe the previously unknown males of a few, recently described, additional species.

## Methods

### Field research

All studied species were collected as adults on their known or putative host-plants. Attempts were also made to obtain larvae to confirm the insect-host plant relationships. Larvae were collected alive by hand using entomological forceps inside flower buds, where they mainly develop during the early flowering season of hosts. Adults destined for morphological analyses were killed in small vials containing cork powder and a few drops of ethylacetate. Additional conspecific adults destined to molecular analyses were killed and preserved in absolute ethanol. Larvae destined for morphological analyses were mostly killed and preserved in a mixture of 75% ethanol and 25% filtered pure water, while additional conspecific specimens destined for molecular analyses were killed and preserved in absolute ethanol. A few larvae, when available in number, destined to SEM analyses, were killed in absolute acetone to preserve the larval cuticular surface and corresponding setae, tubercles, and other projections.

In most cases, adult specimens were collected after direct observations on target plants, with some specimens of possible target beetles directly collected using an aspirator or by hand. This technique had to be employed to avoid physical damage to rare and locally protected plants via netting or beating techniques. Netting or beating were used only for locally abundant host-plants, such as widespread or even invasive species of the *Rubus* or *Prunus* in shrubby habitats. Pollen beetles on these plants are usually highly dispersed and therefore difficult to find and collect using visual observation only.

### Morphological phylogeny and host plant associations

A matrix containing 56 morphological and 5 ecological characters (Tables 3–4) was compiled for all known 63 species of *Meligethes*. The genera *Thymogethes* Audisio and Cline, 2009 (represented here by *T. egenus* from S Europe), *Brassicogethes* Audisio and Cline, 2009 (*B. aeneus* from S Europe), *Tarchonanthogethes* Audisio and Cline, 2009 (*T. fasciatus* from S Africa), *Chromogethes* Kirejtshuk 1989 (*C. splendidulus* from S Africa), *Restiopria* Audisio, Jelínek and Cline, 2011 in [25] (*R. biondii* from S Africa), *Pria* Stephens 1830 (*P. dulcamarae* from S Europe) and *Meligethinus* Grouvelle, 1906 (*M. pallidulus* from W Mediterranean areas) were selected as outgroups based on their different levels of affinities with *Meligethes* Stephens [5–6, 18]. The morphological matrix was produced with MESQUITE version 3.51 [49] and subsequently analyzed in TNT version 1.5 [50]. Multistate characters were treated as unordered and zero-length branches were collapsed. Analyses were run as implicit enumeration under implied weights (concavity factor of 1 and higher), under the “traditional search option”. The following parameters were applied: general RAM of 1 GB, memory set to hold 1 000 000 trees, setting 1000 replicates with tree bisection-reconnection branch swapping and saving 1000 trees per replicate. The most fitting concavity *k*-value of the weighting function was found using the TNT script “setk.run” [51], obtaining a *k* value 10.72. Characters were mapped on one of the most parsimonious trees using Winclada version 1.00.08 [52].

An ancestral state parsimony reconstruction of larval-host-plant associations was carried out in MESQUITE version 3.51 [49] using the most recent phylogeny-based general classification schemes of Rosaceae [35–36]. The reconstruction of ancestral state parsimony for larval-host-plant association (Fig. 9) was carried out and superimposed on our combined morphological and molecular dataset based cladogram following the methods discussed elsewhere [23].

# Insect-host plant relationships and phenology

We collected field information on life history, larval host-plants and phenology on ca. 45 out of 63 species of the genus; for some poorly known and rare species from N Indian subcontinent, China and Japan no additional information on biology was provided. Among the analyzed species, steno-oligophagy (i.e. dependence of a single beetle species on a short series of closely related plant species belonging to the same genus or to closely related genera) is the dominant condition, while monophagous species are few.

Most species of the genus are active during the breeding season in Summer (i.e. middle June to late July; 36 species out of 45, i.e., 80%), while only a couple of mostly W Palaearctic species are Spring specialists (breeding from early April to middle May; 2 species out of 45, i.e. 9%: *M. atratus* and *M. flavimanus*). The remaining 7 analyzed species more widely extend their annual

phenology between May and August.

## Molecular methods

### DNA extraction, amplification and sequencing

A total of 23 adult specimens from 18 different *Meligethes* s.l. species, as well as 4 species of *Brassicogethes* and 2 of *Meligethinus* were collected alive in the field and directly killed and preserved in absolute ethanol. In Table 2, the geographic details for the species are listed. Species identifications were made using morphological characters detailed elsewhere [1, 7–9] and in Tables 1 and 3 in additional files. Total genomic DNA was extracted from whole specimens, following the salting out protocol [53]. Sequences were obtained from two mitochondrial gene fragments, the Cytochrome Oxidase subunit I (COI) and 16S rRNA (16S); and from one nuclear fragment, a portion of the rudimentary gene (CAD). For PCR amplifications, the following primer pairs were used: COI = LC01490 5'-

TCAACAAATCATAAAGATATTGG-3' HC02198 5'-TAAACTTCAGGGTGACCAA AAAATCA-3' [54]; 16S = 16SA5'-  
CGCCTGTTTATCAAAACAT-3'; 16SB 5'- CTCCGGTTGAACTCAGATCA- 3' [55]; CAD = CD439F 5'-  
TTCAGTGTACARTTYCAYCCHGARCAYAC-3' CD688R 5'-TGTATAACCTAGAGGATCDACRTTYTCCATRTTRCA-3' [56].

Amplifications of the mitochondrial genes were performed with the following general cycle conditions: initial denaturation at 96 °C for three minutes, followed by 35 cycles of denaturation at 94 °C for one minute, annealing at 54°–57 °C for 40 seconds, 1-min. extension at 72 °C and a last 7-min. elongation step at 72 °C. Reactions were performed in a 25 µl volume containing (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 16 mM, Tris-HCl 67 mM (pH 8.8 at 25 °C), MgCl<sub>2</sub> 3 mM, 1 mM of each dNTP, 0.8 pmol of each primer and 1.25 units of Taq DNA polymerase. A touchdown PCR protocol was used to amplify the CAD marker with the following thermal cycling conditions: 94 °C for 3.5 min, followed by 20 cycles of 94 °C, 30 s, annealing temperatures stepdowns every cycle of 0.4 °C (from 58 to 50 °C), 35 s, 72 °C, 2.5 min and additional 20 cycles of 94 °C, 30 s, 55 °C, 35 s, 72 °C, 2.5 min. We used an MJ MINI Personal Thermal Cycler (BIO-RAD Laboratories, US) and LifeECO Thermal Cycler to perform PCR amplifications. The PCR products were purified with a GENEAIID-Gel/PCR DNA Fragments Extraction Kit and sent to an external sequencing service (Macrogen Inc.: [www.macrogen.com](http://www.macrogen.com)). Sequences were edited and aligned with GENEIOUS v9.1.6 [57]. A total of 84 new sequences have been deposited in GenBank (Accession numbers xxx-xxx; see Appendix Sx).

### Molecular phylogeny and divergence time estimation

Phylogenetic analyses (BI) were first performed using single-gene alignments and then, Bayesian inference (BI) and Maximum likelihood analysis (ML) were both performed on the concatenated (mtDNA + nucDNA) dataset using, respectively, MRBAYES v3.2.1 [58, 59] and IQ-TREE [60] as implemented in W-IQ-TREE [61]. The best-fit models for the study data sets proved to be a Generalized Time-Reversible model with a proportion of invariable sites and heterogeneous substitution rates following a gamma distribution (GTR + I + G) for the COI and 16S and Generalised Time-Reversible model with a proportion of invariant sites (GTR + I) for CAD. The best fitting model to analyse each partition was selected by jModelTest [44] using the Akaike information criterion. The BI analysis was performed by running 5,000,000 generations, with Markov chains sampled every 1,000 generations. A 10% burn-in was applied and the remaining trees were used to compute a 50% majority rule consensus tree and posterior probabilities. We assessed convergence of the runs by investigating the average standard deviation of split frequencies and effective sample size (ESS) of all parameters in TRACER 1.6 [62]. A value of ESS > 200 was acknowledged as a good indicator of convergence. A ML phylogenetic reconstruction was performed running 1,000 ultrafast bootstrap replications [63] followed by 1,000 replications of assessment of

branch supports with single branch tests with SH-like approximate likelihood ratio test. The best fitting model to analyze each partition was selected as for BI.

To estimate the relative age of lineage divergences, an uncorrelated lognormal Bayesian molecular relaxed clock model and a Yule process prior were used on the mtDNA data set using the software BEAST v.1.8.2 [64]. Given the lack of fossil record or useful dated palaeogeographic records to calibrate trees we used, as a calibration point, an average value of the COI substitution rate in a range between 1.5% and 3.54%, which represent values estimated for mitochondrial DNA in insects [65–68], previously used for closely related groups [6, 23, 65]. Therefore, for the molecular clock analysis we applied an average rate of 0.0126. The GTR model was transferred to the HKY [45] model because there are low ESS values for some parameters in the analyses when applying the GTR model. The analysis was independently performed three times, with 100 million generations and sampling of trees every 10,000 steps. Effective Sample Size (ESS) was evaluated in Tracer v1.6 [62], considering runs with ESS values above 200. Output trees were generated in Tree Annotator v1.8.2 (BEAST package), using maximum clade credibility (MCC) after a 10% burn-in and median heights

## Abbreviations

ARCC – A.R. Cline's collection, housed at the California State Collection of Arthropods in Sacramento, California, USA

BMNH – Natural History Museum, London

CAL – A. Lason's collection, Bialystok, Poland

CAR – P. Audisio's collection, currently housed in the Zoological Museum, Sapienza Rome University, Rome, Italy

CAS – California Academy of Sciences, Sacramento, USA

CHHU – Sadatomo and Sadanari Hisamatsu's Collection, The United Graduate School of Agricultural Sciences, Ehime University, Japan

CSCA – California State Collection of Arthropods, Sacramento, California, USA

ETHZ – Eidgenössische Technische Hochschule, Entomologisches Institut, Zürich, Switzerland

IZAS – Institute of Zoology, Chinese Academy of Sciences, Beijing, China

HNHM – Hungarian Natural History Museum, Budapest, Hungary

MAKB – Zoologische Forschungsinstitut und Museum "Alexander Koenig", Bonn, Germany

MHNG – Muséum d'Histoire Naturelle, Genève, Switzerland

MNHN – Muséum National d'Histoire naturelle, Paris, France

MNST – National Museum of Natural Science, Taichung, Taiwan NHMB – Naturhistorisches Museum, Basel, Switzerland

NHMW – Naturhistorisches Museum, Wien, Austria

NKME – Museum für Naturkunde, Erfurt, Germany

NKMS – Museum für Naturkunde, Stuttgart, Germany

NMPC – National Museum, Prague, Czech Republic

PANW – Institute of Zoology, Polish Academy of Sciences, Warszawa, Poland

RSC – R. Schuh collection, Wiener Neustadt, Austria

SMF – Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt-am-Main, Germany

ZIN – Zoological Institute, Academy of Sciences, St. Petersburg, Russia

ZMUM – Zoological Museum, Moscow State University, Moscow, Russia

ZSM – Zoologische Staatssammlung, München, Germany.

## Declarations

### Acknowledgements

We would like to thank the curators of the following institutions and some private entomologists for loaning valuable specimens necessary to complete our phylogenetic study: J. Jelínek (NMPC), A. Lasoň (CAL), M. Barkley (BMNH), D. Ahrens (MAKB), T. Deuve (MNHN), M. Danielsson (MZLU), F. Hieke (ZMHB), O. Merkl (HNHM), M. Jäch and H. Schilhammer (NHMW), E. Sprecher (NHMB), I. Löbl (MHNG), M. Hartmann (NKME), W. Schwaller (NKMS), R. Schuh (RSC), D. Kovac (SMF), A.G. Kirejtshuk (ZIN), and N. B. Nikitsky (ZMUM). Special thanks are due to our colleague Sadatomo Hisamatsu (Matsuyama, Japan), for his valuable help in providing us with precious material of *Meligethes* spp. from Japan and Taiwan. We greatly appreciated the valuable comments on an earlier version of this manuscript received from our dear friend and colleague Josef Jelínek, Department of Entomology, National Museum, Horní Počernice, Czech Republic. The authors greatly thank also the colleague Kirill Makarov (Moscow, Russia) for authorization to use his picture of *Meligethes flavimanus* (Fig. 1 A (a)), as well as our friend Lec Borowiec (Wrocław, Poland) for authorization to use his picture of *Odonthogethes denticulatus* (Fig. 1 A (b)). Special thanks are also due to N. Falchi (Rome), for preparation of the Fig. 1 A (c)). The authors are grateful to Prof. Xinfen Gao, Curator of the Herbarium (CDBI), Chengdu Institute of Biology, Chinese Academy of Sciences, and to the Chinese amateur botanist Shuanlu Dong (Meixian, Shaanxi) for cooperation and assistance in identifying several host plants.

### Authors' contributions

PA, SS, ML and MH designed the project, SS, PA, DB, ML, EM, SP and AS analyzed the data and prepared graphs and illustrations, PA, ARC, SS, MT, ML, DB and EM wrote the manuscript, PA, ML, and MH contributed materials and resources. All authors contributed to the final manuscript.

### Funding

This paper was partly supported by funds to PA and SS from the Italian Ministero dell'Università e della Ricerca. This paper was also partly supported by funds from the Fundamental Research Funds for Chinese Central Universities (Z109021305), and the senior author (ML) thanks the China Scholarship Council for financial support for her stay in Rome (November, 2016–December, 2017), in collaboration with coauthor P.A.

### Availability of data and materials

Sequence data (mtDNA and nDNA) can be found in GenBank (see above under Additional files). Material of Meligethinae studied for the aims of the present research was examined from or is preserved in the institutions and private collections listed above under Abbreviations.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare to have not competing interests.

## References

1. A new fossil tortricid

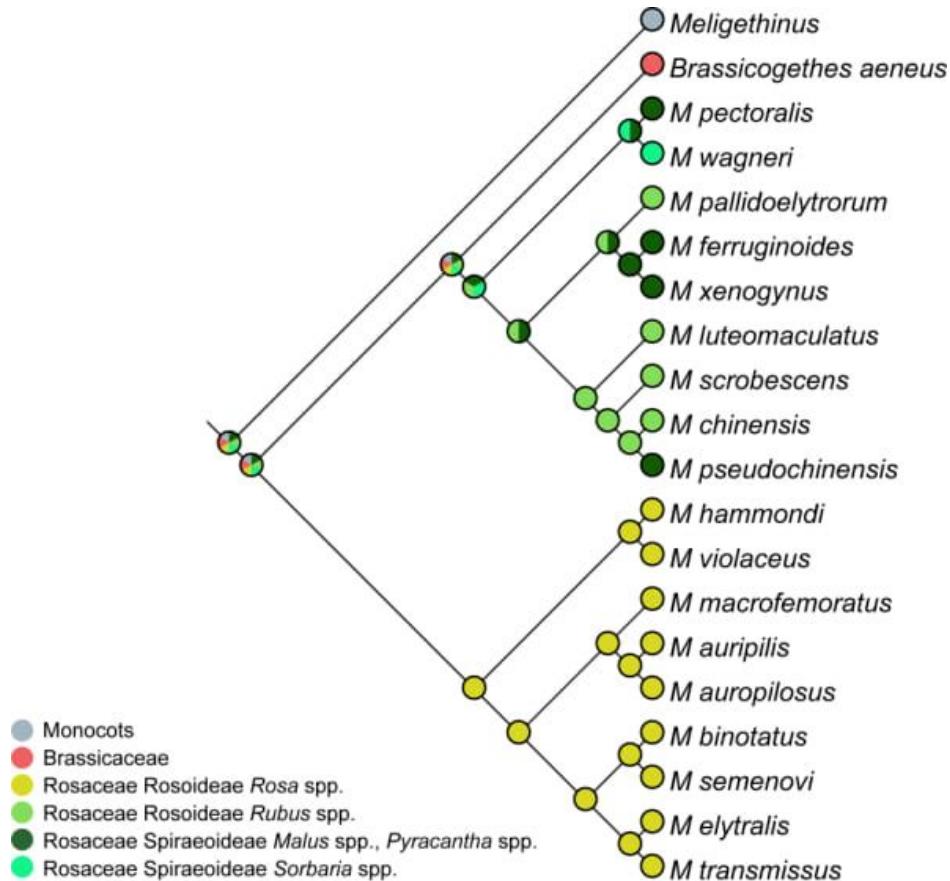
- Poinar G, Jr, Brown JW. 1993. A new fossil tortricid.
2. (Lepidoptera. Tortricidae) from Dominican amber. Entomol.
  3. Scand. 23, 25–29.
  4. Audisio P Coleoptera. Nitidulidae - Kateretidae. Fauna d'Italia. Bologna: Calderini Edizioni; 1993.
  5. Audisio P, Cline AR, De Biase A, Antonini G, Mancini E, Trizzino M, Costantini L, Strika S, Lamanna F, Cerretti P. Preliminary re-examination of genus-level taxonomy of the pollen beetle subfamily Meligethinae (Coleoptera: Nitidulidae). Acta Ent Mus N Pragae. 2009;49:341–504.
  6. Jelínek J, Carlton CE, Cline AR, Leschen RAB. 10.26. Nitidulidae Latreille. Berlin: Walter De Gruyter; 2010.
  7. Cline AR, Smith TR, Miller K, Moulton M, Whiting M, Audisio P. Molecular phylogeny of Nitidulidae: assessment of subfamilial and tribal classification and formalization of the family Cybocephalidae (Coleoptera: Cucujoidea). Syst Ent. 2014;39:758–72.
  8. Liu M, Sabatelli S, Mancini E, Trizzino M, Huang M, Cline AR, Audisio P. Rediscovery of Brassicogethes salvan (Coleoptera: Nitidulidae, Meligethinae) in the southwestern Alps. Ins Cons Div. 2020;12:80–7.
  9. Liu M, Huang M, Cline AR, Cardoli P, Audisio P, Sabatelli S. Re-examination of the genus-level taxonomy of the pollen beetle subfamily Meligethinae – Part 1. Sagittogethes Audisio & Cline 2009 and allied genera; with description of a new genus (Coleoptera: Nitidulidae). Fragm entomol. 2020;52(1):119–35.
  10. Audisio P, Sabatelli S, Jelínek J. Revision of the pollen beetle genus Meligethes (Coleoptera: Nitidulidae). Fragm entomol. 2015;46:19–12.
  11. Liu M, Yang X, Huang M, Jelínek J, Audisio P. Four new species of Meligethes Stephens from China and additional data on other species of the genus (Coleoptera: Nitidulidae: Meligethinae). Zootaxa. 2016;4121:101–16.
  12. Liu M, Huang M, Cline AR, Sabatelli S, Audisio P. A new species of Meligethes Stephens from China and additional data on members of the *M. chinensis* species-complex (Coleoptera: Nitidulidae, Meligethinae). Fragm entomol. 2017;49:79–84.
  13. Liu M, Huang M, Cline AR, Audisio P. New and poorly known Meligethes Stephens from China, with bionomical data on some species (Coleoptera: Nitidulidae: Meligethinae). Zootaxa. 2018;4392:546–66.
  14. Ormerod EA. Life history of Meligethes. Ent mon Mag. 1874;11:46–52.
  15. Hayashi N. A contribution to the knowledge of the larvae of Nitidulidae occurring in Japan (Coleoptera: Cucujoidea). Ins Matsum. 1978;14:1–97.
  16. Hisamatsu S-T. Revision of the Meligethinae of Taiwan (Coleoptera, Nitidulidae). Japan J Syst Ent. 2009;15:17–46.
  17. Audisio P, De Biase A, Romanelli P, Angelici MC, Ketmaier V, De Matthaeis E. Molecular re-examination of the taxonomy of the *Meligethes viridescens* species complex (Coleoptera: Nitidulidae). Biochem Syst Ecol. 1999;28:1–13.
  18. Audisio P, Belfiore C, De Biase A, Antonini G. Identification of *Meligethes matronalis* Audisio and Spornraft, 1990 and *M. subaeneus* Sturm, 1845, based on morphometric and bionomic characters (Coleoptera: Nitidulidae). Eur J Ent. 2001;98:87–97.
  19. Audisio P, De Biase A, Antonini G, Belfiore C, Oliverio M. Morphological, molecular, and ecological evidence of a new Euro-Anatolian species of the *Meligethes coracinus* complex (Coleoptera: Nitidulidae). Ins Syst Evol. 2001;31:361–85.
  20. Audisio P, De Biase A, Antonini G, Oliverio M, Ketmaier V, De Matthaeis E. Specific distinction by allozymic data of sympatric sibling species of the pollen beetle genus *Meligethes* (Coleoptera: Nitidulidae). It J Zool. 2002;69:65–9.
  21. Audisio P, De Biase A, Antonini G. A new exceptional *Meligethes* of the *M. aeneus* species-group from Western Alps and an updated key to identification of *M. aeneus* and allied species (Coleoptera: Nitidulidae: Meligethinae). Ins Syst Evol. 2003;34:121–30.
  22. Audisio P, De Biase A, Antonini G, Mancini E, Özbek H, Gultekin L. Redescription and natural history of *Meligethes longulus* Schilsky, 1894, and provisional revision of the *M. coracinus* species-complex (Coleoptera, Nitidulidae, Meligethinae). It J Zool. 2005;72:73–85.
  23. Audisio P, Mancini E, De Biase A. A new species of the pollen-beetle genus *Meligethes* (Coleoptera: Nitidulidae) of the *M. aeneus* group from Greece, with review of the *M. subaeneus* complex. Zootaxa. 2006;1275:43–60.
  24. Audisio P, Cline AR, Mancini E, Trizzino M, Avgin SS, De Biase A. Four new Palaearctic *Brassicogethes* (Coleoptera, Nitidulidae, Meligethinae), and phylogenetic inference on the *B. coracinus* group. Rend Lincei. 2011;22:235–68. Doi:10.1007/s12210-011-0126-4.

25. De Biase A, Antonini G, Mancini E, Trizzino M, Cline A, Audisio P. Discordant patterns in the genetic, ecological, and morphological diversification of a recently radiated phytophagous beetle clade (Coleoptera: Nitidulidae: Meligethinae). *Rend Lincei*. 2012;23:207–15.
26. Mancini E, De Biase AD, Cline AR, Antonini G, Trizzino M, Clayhills T, Sabatelli S, Cerretti P, Audisio P. Morphological, genetic and host-plant diversification in pollen-beetles of the *Brassicogethes coracinus* group (Coleoptera: Nitidulidae: Meligethinae). *Rend Lincei*. 2016;27:321–39.
27. Trizzino M, Audisio P, Antonini G, De Biase A, Mancini E. Comparative analysis of sequences and secondary structures of the rRNA internal transcribed spacer 2 (ITS2) in pollen beetles of the subfamily Meligethinae (Coleoptera, Nitidulidae): Potential use of slippage-derived sequences in molecular systematics. *Mol Phyl Evol*. 2009;51:215–26.
28. Audisio P, Jelínek J, Cline AR, Mancini E, Trizzino M, Cerretti P, Antonini G. Description and taxonomic position of a new genus and species of southern African pollen beetle (Coleoptera: Nitidulidae: Meligethinae). *Zootaxa*. 2011;2927:49–56.
29. Audisio P, Cline AR, Solano E, Mancini E, Lamanna F, Antonini G, Trizzino M. A peculiar new genus and species of pollen-beetle (Coleoptera, Nitidulidae) from eastern Africa, with a molecular phylogeny of related Meligethinae. *Syst Biodiv*. 2014;12:77–91.
30. Stephens JF. Illustrations of British Entomology; or, a Synopsis of Indigenous Insects: containing their generic and specific distinctions; with an account of their metamorphoses, times of appearance, localities, food, and economy, as far as practicable. Mandibulata. 1830; Baldwin & Cradock, London.
31. Olivier AG. Entomologie, ou histoire naturelle des insectes, avec leurs caractères génériques et spécifiques, leur description, leur synonymie, et leur figure enluminée. Coleoptères. Tome second. 1790;de Baudouin, Paris.
32. Marsham T. Entomologia Britannica, sistens insecta britanniae indigena, secundum methodum linnaeanam disposita. Tomus I. Coleoptera. 1802;Wilks et Taylor, Londini.
33. Heer O. Fauna Coleopterorum Helvetica. Pars 1 (3). 1841;Orelii, Fuesslini et Sociorum, Turici:361–652.
34. Rebmann O. Revision der Gattung Meligethes (Col. Nitid.). Die paläarktischen Arten der Gruppe des *M. atratus* Ol. (7. Beitrag zur Kenntnis der Nitiduliden.). *Ent Bl Zeitschr Biol Syst Käf*. 1956;52:124–35.
35. Rebmann O. Revision der Gattung Meligethes Subgenus Odonthogethes (Col. Nitid.) (6. Beitrag zur Kenntnis der Nitiduliden). *Ent Bl Zeitschr Biol Syst Käf*. 1956;52:42–8.
36. Chen Y, Kirejtshuk AG, Huang M. A new species of the subgenus Meligethes Stephens (Coleoptera: Nitidulidae) from Sichuan, China. *Entomotaxonomia*. 2013;35:35–40.
37. Chen Y, Lin X, Huang M, Yang XK. A new species of Lamiogethes and a new species of Meligethes from China (Coleoptera: Nitidulidae: Meligethinae). *Zootaxa*. 2015;3999:413–20.
38. Potter D, Eriksson T, Evans RC, Oh S, Smedmark JEE, Morgan DR, Kerr M, Robertson KR, Arsenault M, Dickinson TA. Phylogeny and classification of Rosaceae. *Plant Syst Evol*. 2007;266:5–43.
39. Potter D, Still SM, Grebenc T, Ballian D, Božič G, Franjiæ J, Kraigher H. Phylogenetic relationships in tribe Spiraeae (Rosaceae) inferred from nucleotide sequence data. *Plant Syst Evol*. 2007;266:105–18.
40. Audisio P, Cline AR, Lamanna F, Trizzino M, Antonini G, Mancini E, De Biase A. Revision of the southern African pollen beetle genus *Anthystrix* (Coleoptera: Nitidulidae: Meligethinae). *Ann Ent Soc Am*. 2009;102:998–1012.
41. Audisio P, Cline AR, Trizzino M, Mancini E, Antonini G, Sabatelli S, Cerretti P. Revision of the African pollen beetle genera *Tarchonanthogethes* and *Xenostrongylogethes*, with insect-host plant relationships, identification key, and cladistic analysis of the *Anthystrix* genus-complex (Coleoptera: Nitidulidae: Meligethinae). *Zootaxa*. 2015;3920:101–52.
42. Alice LA, Campbell CS. Phylogeny of Rubus (Rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. *Am J Bot*. 1999;86:81–97.
43. Fougère-Danezan M, Joly S, Bruneau A, Gao XF, Zhang LB. Phylogeny and biogeography of wild roses with specific attention to polyploids. *Ann Bot London*. 2014;115:275–91.
44. Wang Y, Chen Q, Chen T, Tang H, Liu L, Wang X. Phylogenetic Insights into Chinese Rubus (Rosaceae) from Multiple Chloroplast and Nuclear DNAs. *Front Pl Sci*. 2016;7:968.
45. Lu LD, Gu CZ, Li CL, Crinan A, Bartholomew B, Brach AR, Boufford DE, Ikeda H, Ohba H, Robertson KR, Spongberg SA. Rosaceae. In: Wu ZY, Ravan PH, Hong DY, editors. *Flora of China*. Vol. 9. Beijing/St. Louis: Science Press/Missouri Botanical Garden Press; 2003. pp. 46–34.

46. Rodríguez F, Oliver JL, Marín A, Medina JR. The general stochastic model of nucleotide substitution. *J Theor Biol.* 1990;142:485–501.
47. Posada D. jModelTest: phylogenetic model averaging. *Mol Phyl Evol.* 2008;25:1253–56.
48. Hasegawa M, Iida Y, Yano TA, Takaiwa F, Iwabuchi M. Phylogenetic relationships among eukaryotic kingdoms inferred from ribosomal RNA sequences. *J Mol Evol.* 1985;22(1):32–8. <https://doi.org/10.1007/BF02105802>.
49. Sabatelli S, Liu M, Cline AR, Lasoń A, Macuvele S, Muambalo K, Chuquela L, Audisio P. Palms and pollen Beetles: two new anthophilous beetle species of Meligethinus from Mozambique (Coleoptera: Nitidulidae: Meligethinae). *Zootaxa.* 2020;4802(1):032–40.
50. Xiang Y, Huang CH, Hu Y, Wen J, Li S, Yi T, Chen H, Xiang J, Ma H. Evolution of Rosaceae Fruit Types Based on Nuclear Phylogeny in the Context of Geological Times and Genome Duplication. *Mol Biol Evol.* 2016(2017);34:262–81.
51. Fang JY, Song J-C, Liu H-J, Piao S-L. Vegetation-Climate relationship and its application in the division of Vegetation Zones in China. *Acta Bot Sinica.* 2002;44:1105–22.
52. Maddison WP, Maddison DR. Mesquite: A modular system for evolutionary analysis, version 2.74. 2010;<http://mesquiteproject.org>.
53. Goloboff PA, Catalano SA. TNT version 1.5, including a full implementation of phylogenetic morphometrics[J]. *Cladistics.* 2016;32:221–38.
54. Santos BF, Payne A, Pickett KM, Carpenter JM. Phylogeny and historical biogeography of the paper wasp genus *Polistes* (Hymenoptera: Vespidae): implications for the overwintering hypothesis of social evolution. *Cladistics.* 2015;31:535–49.
55. Nixon KC. Winclada. Version 1.00.08. Published by the Author, Ithaca, New York. [WWW document]. 2002;<http://www.cladistics.com>. [accessed on January 2017, no longer available].
56. Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucl Ac Res.* 1997;25:4692–93.
57. Folmer O, Black MB, Wr H, Lutz R, Vrijenhoek RC. DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Mol mar biol biotechn.* 1994;3(5):294–9.
58. Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann ent Soc Am.* 1994;87:651–701.
59. Wild AL, Maddison DR.. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. *Mol Phyl Evol.* 2008;48:877–91.
60. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics.* 2012;28:1647–49. Doi:10.1093/bioinformatics/bts199.
61. Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics.* 2001;17:754–5.
62. Brower AVZ. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA Evolution. *PNAS.* 1994;91:6491–5.
63. Nguyen L-T, Schmidt HA, Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol.* 2015;32:268–74.
64. Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucl Ac Res.* 2016;44(W1):W232–5.
65. Rambaut A, Suchard M, Xie D, Drummond A. Tracer v1. 6. 2014;Retrieved from <http://tree.bio.ed.ac.uk/software/tracer>.
66. Minh BQ, Nguyen MAT, von Haeseler A. Ultrafast approximation for phylogenetic bootstrap. *Mol Biol Evol.* 2013;30:1188–95.
67. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol.* 2012;29:1969–73.
68. Sabatelli S, Liu M, Badano D, Mancini E, Trizzino M, Cline AR, Endrestøl A, Huang M, Audisio P. Molecular phylogeny and host-plant use (Lamiaceae) of the Thymogethes pollen beetles (Coleoptera). *Zool Scr.* 2020;49:28–46.
69. Farrell BD. Evolutionary assembly of the milkweed fauna: Cytochrome oxidase I and the age of Tetraopes beetles. *Mol Phyl Evol.* 2001;18:467–78.

70. Papadopoulou A, Anastasiou I, Vogler AP. Revisiting the Insect Mitochondrial Molecular Clock: The Mid-Aegean Trench Calibration. *Mol Phyl Evol*. 2010;27:1659–72.
71. Pons J, Ribera I, Bertranpetti J, Balke M. Nucleotide substitution rates for the full set of mitochondrial protein-coding genes in Coleoptera. *Mol Phyl Evol*. 2010;56:796–807.
72. Evenhuis NL. The Insect and Spider Collections of the World Website. 2020; <http://hbs.bishopmuseum.org/codens/> [accessed at March 26th, 2020].
73. CAPTIONS.

## Figures



**Figure 1**

The strict consensus tree based on combined morphological and molecular dataset constructed via MrBayes, superimposed on ancestral state parsimony reconstruction of larval-host-plant associations executed in MESQUITE (tribes within Rosaceae according to [35–36, 40, 47]).

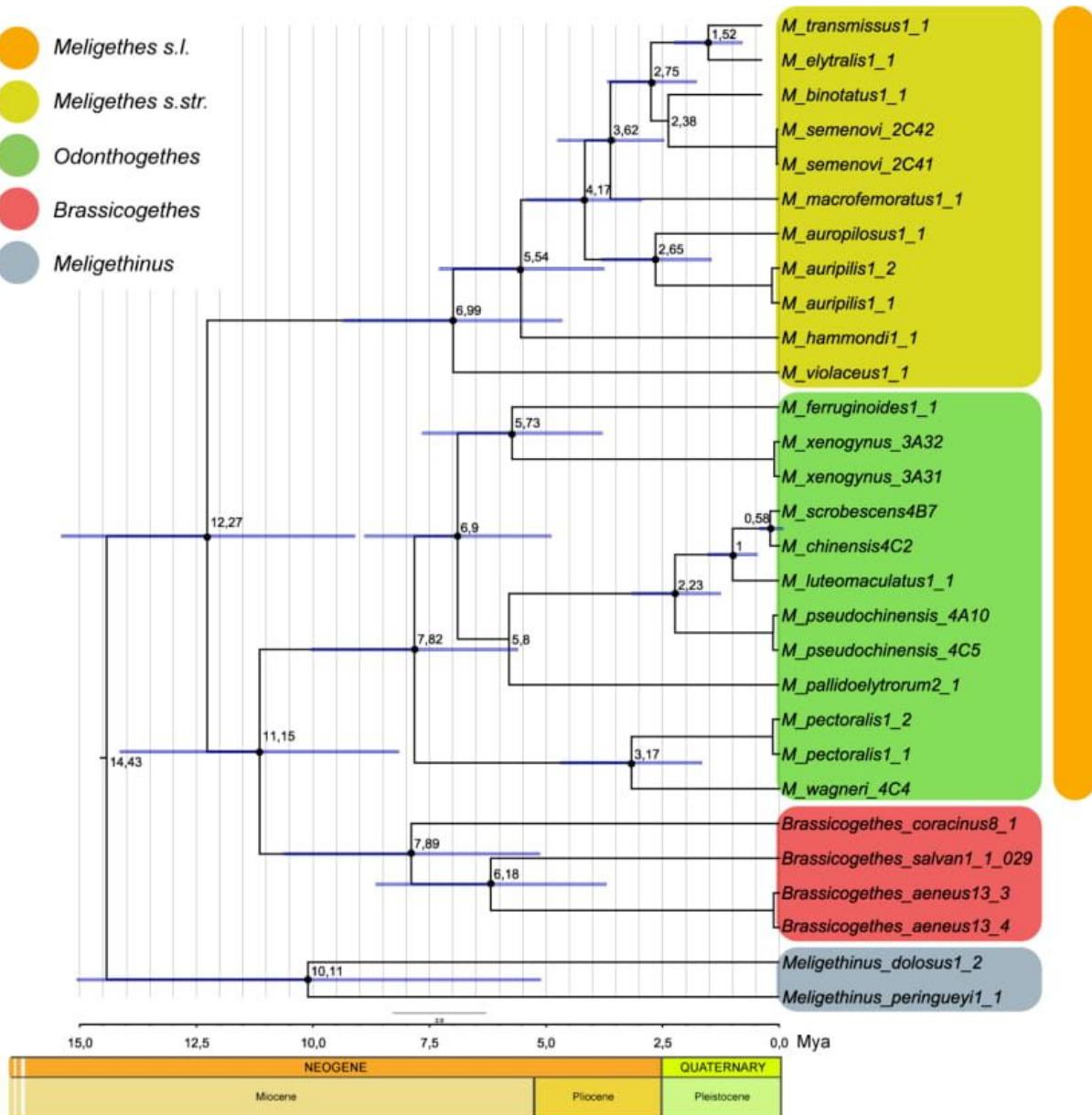


Figure 2

Time-calibrated BEAST phylogeny of representative members of *Meligethes* s.str., *Odonthogethes*, *Brassicogethes* and *Meligethinus*, inferred from combined mitochondrial sequences (COI, 16S). Numbers at nodes correspond to estimated age (Mya) obtained with calibration of 0.0126 substitutions/site per My; bars represent highest posterior densities (95%) around mean date estimates. Nodes with black dots were supported with high posterior support (>95%).

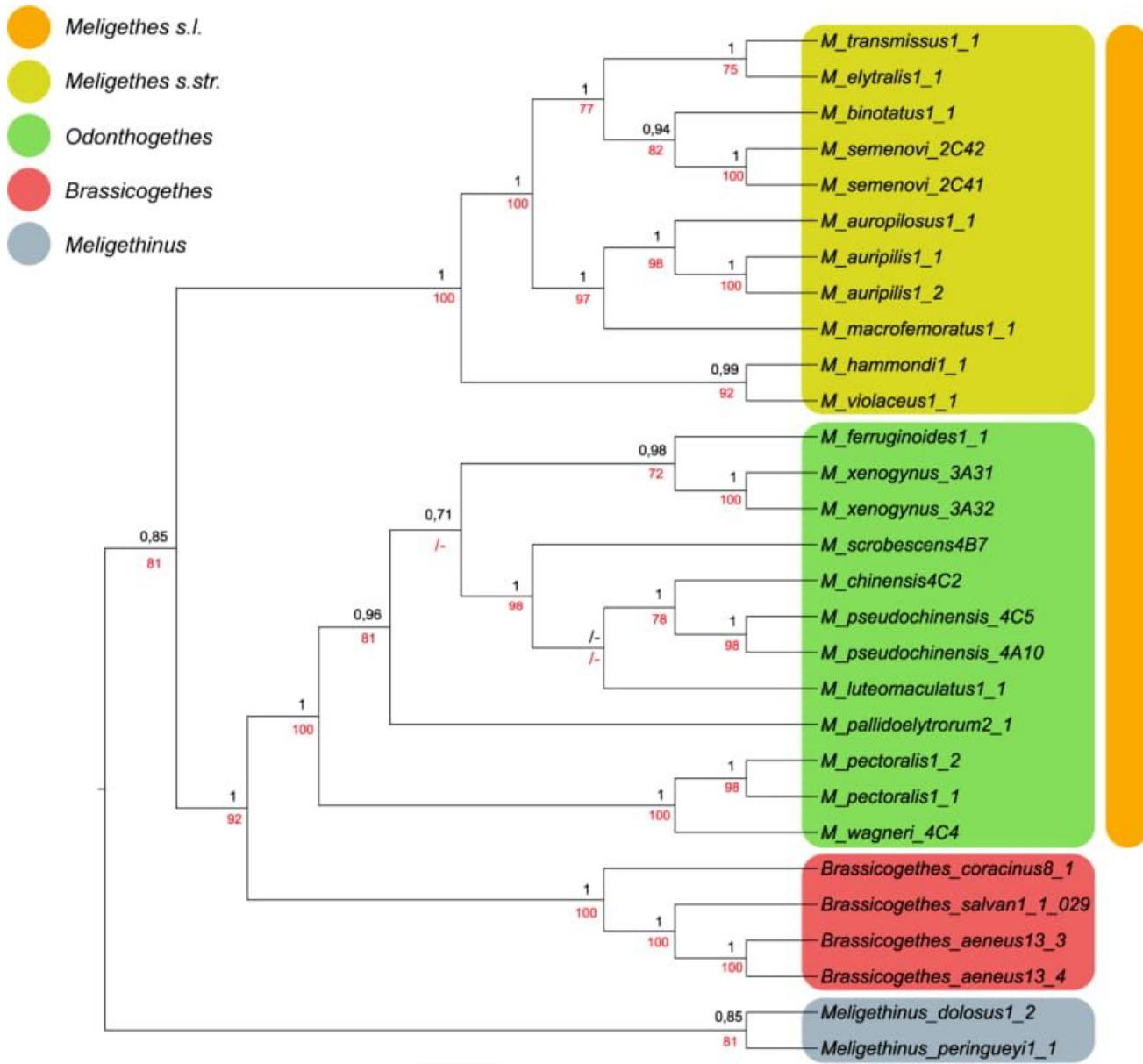
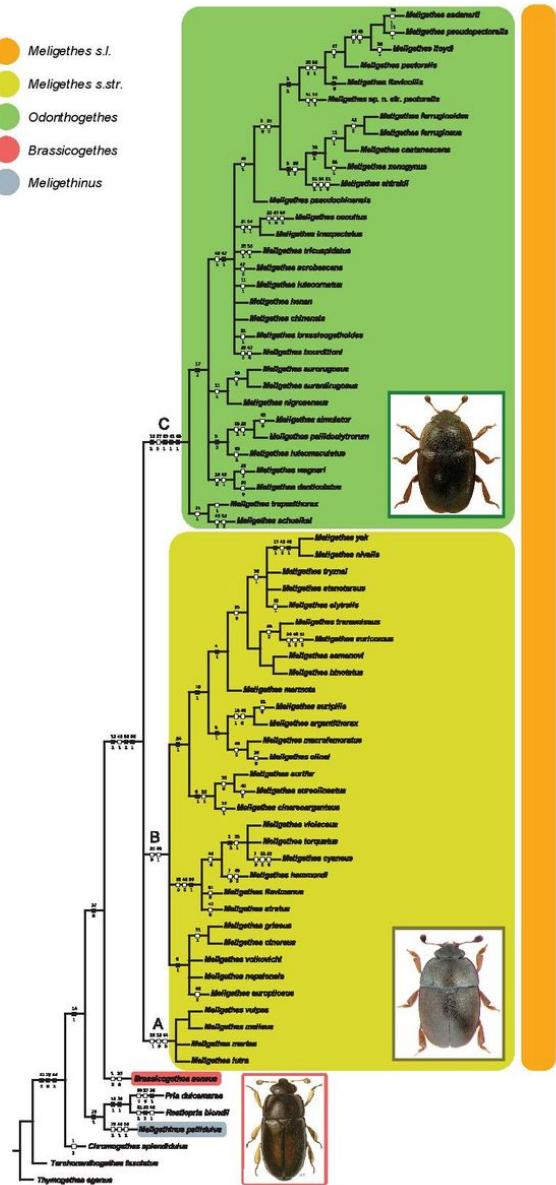


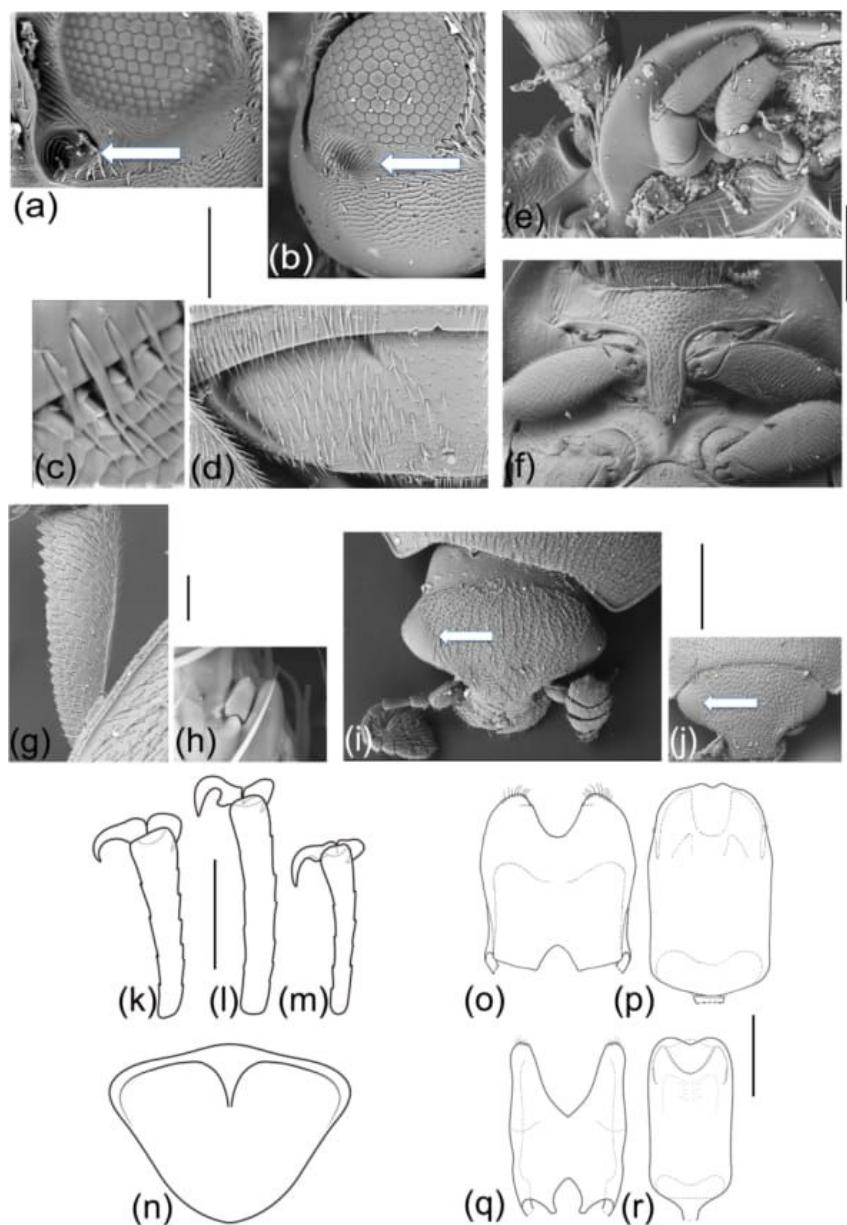
Figure 3

Phylogenetic interrelationships of representative members of Meligethes (s.l.) (Meligethes s.str. + Odonthogethes), Brassicogethes, and Meligethinus based on the concatenated molecular dataset (COI, 16s, CAD) using Bayesian inference (BI) performed by MrBayes, and maximum likelihood (ML) analyses performed by IQ-TREE. The final molecular data matrix includes 29 terminals and 1841 aligned characters. See Table 2, for details on the examined specimens. Only BI posterior probability (black) values and ML bootstrap (red) values exceeding 70% are shown.



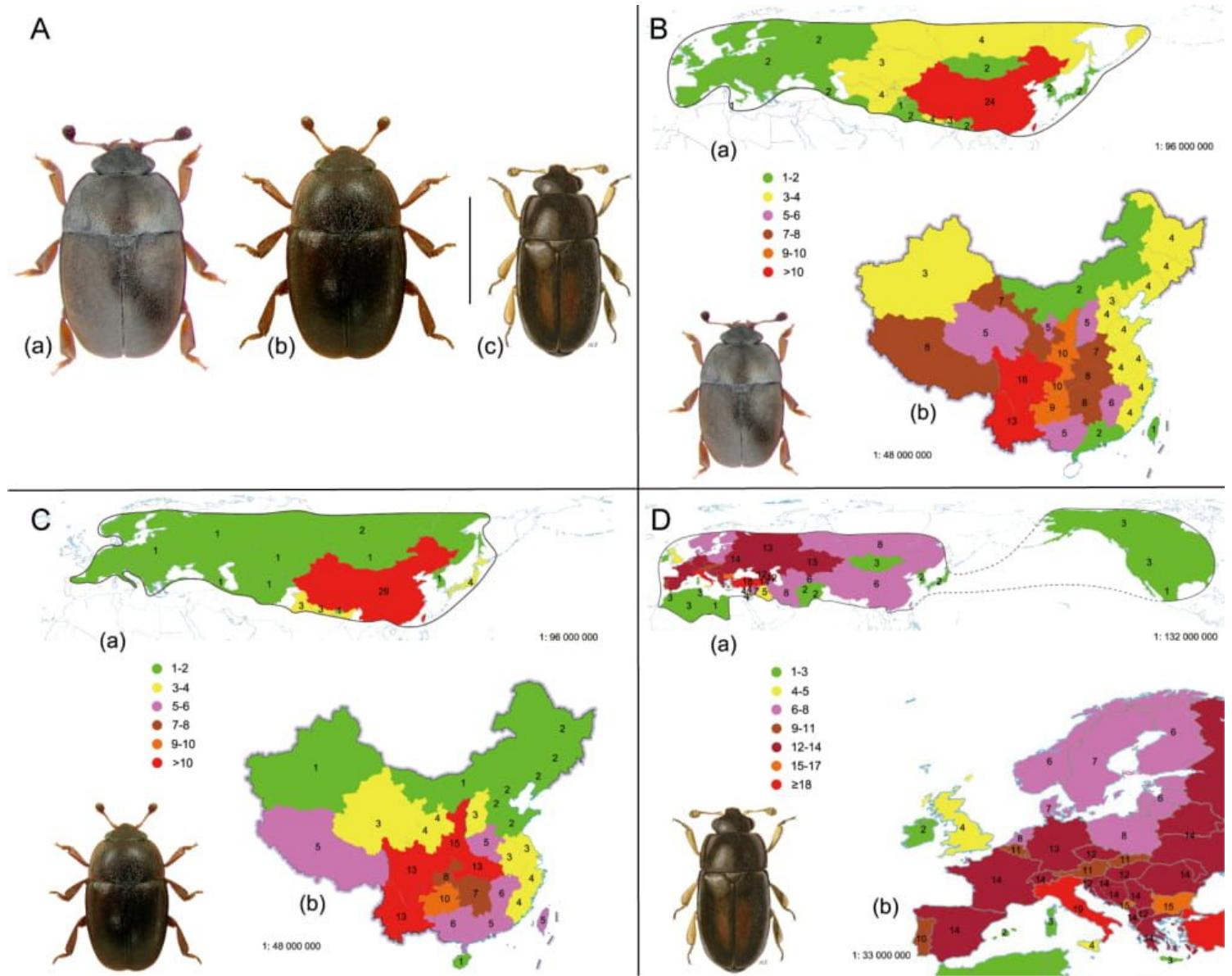
**Figure 4**

One of the most parsimonious trees based on morphological data (tree length = 160, C.I. = 0.42, R.I. = 0.83). Numbers in boxes under branches indicate Bremer support values. Apomorphic and plesiomorphic character states are indicated with black and empty squares, respectively. Capital letters refer to clades as determined by morphological cladistic analysis (see Results and Discussion); the present-day subgeneric classification is superimposed on the C (*Odontogethes*) and A+B (*Meligethes* s.str.) clades. Reconstruction is based on 56 morphological characters (128 states) for 63 members of *Meligethes*, + *Brassicogethes aeneus*, *Meligethinus pallidulus*, *Pria dulcamarae*, *Restiopria biondii*, *Tarchonanthogethes fasciatus*, *Chromogethes splendidulus*, and *Thymogethes egenus* (outgroups) (see Tables 3 and 4 in additional files for character list and matrix).



**Figure 5**

SEM pictures of *Meligethes* (s.l.) spp.; (a) left eye and ventral portion of temples (*Meligethes flavimanus*); (b) left eye and lateral portion of temples (*Odonthogethes castanescens*). Arrows indicate shared pit in ventral subocular lateral portions of temples (inside terminal posterior portion of ventral furrows in members of subgenus *Meligethes* s. str., laterad and more dorsad in members of *Odonthogethes*). (c) Microsetae along posterior edge of pronotum of *Odonthogethes castanescens*. (d) Left portion of last visible abdominal ventrite of *Odonthogethes denticulatus*. (e) Right maxillary palp and labial palp of *Odonthogethes denticulatus*. (f) Prosternum, prosternal process and mesoventrite of *Odonthogethes denticulatus*. (g) Left protibia, dorsal view, of *Meligethes atratus*. (h) Close-up of left protibia, dorsal view, of *Odonthogethes castanescens*, with pre-distal tooth and spicule. Dorsal head view in *Meligethinae*; (i) *Meligethes atratus*; (j) *Brassicogethes aeneus*. Arrows of Figs (i) and (j) point to the shared absence of dorsal circumocular furrows on dorsal head surface. Drawings of *Meligethes* (s. l.) spp. (k-r); terminal left metatarsomeres: (k) *Meligethes atratus*; (l) *Odonthogethes denticulatus*; (m) *Odonthogethes flavicollis*. (n) Last visible dorsal abdominal segment (pygidium) of *Odonthogethes flavicollis*. Male genitalia (tegmen and median lobe of aedeagus) in *Meligethes* (s. l.) and *Brassicogethes*; (o, p) *Meligethes* (M.) *atratus*; (q, r) *Brassicogethes salvan* (Audisio, Antonini & De Biase, 2003). Scale bar = 0.01 mm (Fig. h); = 0.02 mm (Fig. c); = 0.1 mm (Figs e, g, k-m); = 0.12 mm (Figs a, b); = 0.18 mm (Fig. d); = 0.2 mm (Figs o-r); = 0.4 mm (Figs i, j); = 0.5 mm (Figs f, n).



**Figure 6**

A *Meligethes* complex of genera; (a) *Meligethes flavimanus* Sturm, 1845 from Russia, E Siberia (photo by Kirill Makarov); (b) *Odonthogethes denticulatus* (Heer, 1841) from Poland (photo by Lech Borowiec); (c) *Brassicogethes longulus* (Schilsky, 1894) from E Turkey (color drawing by Niccolò Falchi). Scale bar = 1.5 mm. B (a) Map of *Meligethes* s.str. species richness throughout their overall geographic range. Geographic units are all defined political countries, except for the Asiatic portion of the Russian Federation (the main geographic subdivisions are considered separately), and China (all China is represented). European and Caucasian countries, where two widespread species are present, are considered together. (b) Map of *Meligethes* s.str. species richness in China (each administrative province and Taiwan are considered separately). C (a) Map of *Odonthogethes* species richness throughout their overall geographic range [same criteria as in Fig. 1B(a)]. European and Caucasian countries, where only one widespread species is present, are considered together. (b) Map of *Odonthogethes* species richness in China. D (a) Map of *Brassicogethes* species richness throughout their overall geographic range [same criteria as in Fig. 1B(a)]. Nearctic countries are considered together. (b) Map of *Brassicogethes* species richness in Europe and in Mediterranean areas (each country and the main, distinct geographical units are considered separately). Numbers of each *Meligethes*, *Odonthogethes* and *Brassicogethes*, species are reported for each country, main geographic subdivision or administrative unit. Countries with absence of the studied species are represented by no color.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx
- Additionalfile3Table4.xlsx
- Additionalfile2Table3.docx
- Additionalfile4.pdf
- Additionalfile5.pdf
- Additionalfile6.pdf
- Additionalfile7.pdf