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Research Article

# *Andricus pseudocecconii* sp. nov. (Hymenoptera: Cynipidae: Cynipini) from Iran

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**Abstract**
A new species of oak gall wasp, *Andricus pseudocecconii* Melika, Tavakoli & Stone, sp. nov. (Hymenoptera: Cynipidae, Cynipini) is described. Descriptions, diagnoses, biology, and host associations for the new species are given. The new taxon is supported by morphological and molecular data.

**Key words:** *Andricus*, *Quercus*, biology, cynipids, diagnosis, distribution.

# گزارش گونه جدید زنبور *Andricus pseudocecconii* sp. nov. (Hymenoptera: Cynipidae: Cynipini) از ایران

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**چکیده**
گونهٔ جدید زنبورگالزای بلوط با نام، *Andricus pseudocecconii* Melika, Tavakoli & Stone, **sp. nov**. (Hymenoptera: Cynipidae, Cynipini) توصیف شده است. توضیحات مربوط به شکل‌شناسی، تشخیص، زیست شناسی و میزبان مرتبط این گونه جدید ارایه شده است. موقعیت این تاکسون جدید توسط داده‌های ریخت‌شناسی و مولکولی مورد تایید قرار گرفته است.

**واژه‌های کلیدی:** *Andricus*، زنبورهای گالزا، زیست شناسی، تشخیص، پراکنش

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## Introduction

The Western Palaearctic fauna of oak gall wasps (Hymenoptera, Cynipidae, Cynipini) comprises 161 species in 12 genera (Pénzes *et al.,* 2018). The most species–rich tribe within the family Cynipidae in Iran is the Cynipini (oak gall wasps), with 74 species in 11 genera, and with the highest species richness in the central and northern Zagros (Tavakoli *et al*., 2021). The few studies on Iranian cynipids before 1980 was summarised by Chodjai (1980). Little was added before 2000, but since then considerable advances have been made. This is particularly true for the oak gall wasps, with many new species described (Melika & Stone, 2001; Melika *et al*., 2004; Azizkhani *et al*., 2006; Tavakoli *et al*., 2008; Sadeghi *et al*., 2009; Melika *et al*., 2011; Pujade-Villar *et al*., 2015). In addition, many new species have been described from Turkey, the neighboring geography of Iran (Azmaz & Katilmis, 2017, 2020a, 2020b, 2021a, 2021b; Demirel *et al*., 2022; Mutun *et al*., 2014, 2020). Here we describe a new species, *Andricus pseudocecconii* Melika, Tavakoli & Stone, **sp. nov**., whose existence was suspected based on DNA sequence divergence and bimodal variation in gall structure within the known species *Andricus cecconii* Kieffer, 1901 (Tavakoli *et al*., 2019).

## Materials and methods

### Morphological descriptions

The terminology used to describe gall wasp morphology follows other recent cynipid studies (Harris, 1979; Melika, 2006; Melika *et al*., 2010). Measurements and abbreviations used here include: F1–F12 for the 1st and subsequent flagellomeres; POL (post-ocellar distance) for the distance between the inner margins of the posterior ocelli; OOL (ocellar-ocular distance) for the distance from the outer edge of a posterior ocellus to the inner margin of the compound eye; and LOL (lateral-frontal ocelli distance) for the distance between lateral and frontal ocelli. The width of the forewing radial cell is measured from the margin of the wing to the Rs vein.

Bright field images of adults were produced with a digital Leica DC500 camera attached to a Leica DM2700M compound microscope using the LAS Store & Recall software, followed by processing in Adobe Photoshop 6.0. Gall images were taken in the field by M. Tavakoli.

The type material is deposited in PHDNRL, Plant Health Diagnostic National Reference Laboratory, National Food Chain Safety Office, Budapest, Hungary.

### Molecular techniques

DNA sequence data were used to corroborate the patterns in adult and gall morphology and confirm the status of the new species. Sections of two genes were sequenced, a 433 base pair section of the mitochondrial cytochrome *b* (cytb) gene and the nuclear second rRNA internal transcribed spacer region (ITS2; approximately 496 base pairs), both of which are known to be informative for delimiting oak gall wasp species (see Nicholls *et al*., 2012, 2018a, 2018b; Cerasa *et al*., 2020). Data were generated for multiple individuals of *A. pseudocecconii* and its close relative *A. cecconii* (including the latter species' asexual generation, previously known as *A. megalucidus*), and combined with previously published data for these two taxa (Tavakoli *et al*., 2019). In addition, individuals of five other species from the same clade of *Andricus* species were included to provide some phylogenetic context with close relatives. Alignments were made using MAFFT version 7.471 (Katoh & Standley, 2013), and maximum likelihood trees for each gene subsequently constructed using the web-interface version of IQTREE (Nguyen *et al*., 2015) using gene-specific substitution models (HKY+F+I for cytb, TPM3+F for ITS2) automatically selected by ModelFinder (Kalyaanamoorthy *et al*., 2017). All new sequences have been deposited in GenBank, accession numbers OL957051-OL957089 (cytb) and OL963980-OL964017 (ITS2).

## Results

Below we provide a detailed description of adults and galls of the new species, with data on their biology and distribution.

***Andricus pseudocecconii* Melika, Tavakoli & Stone, sp. nov.**

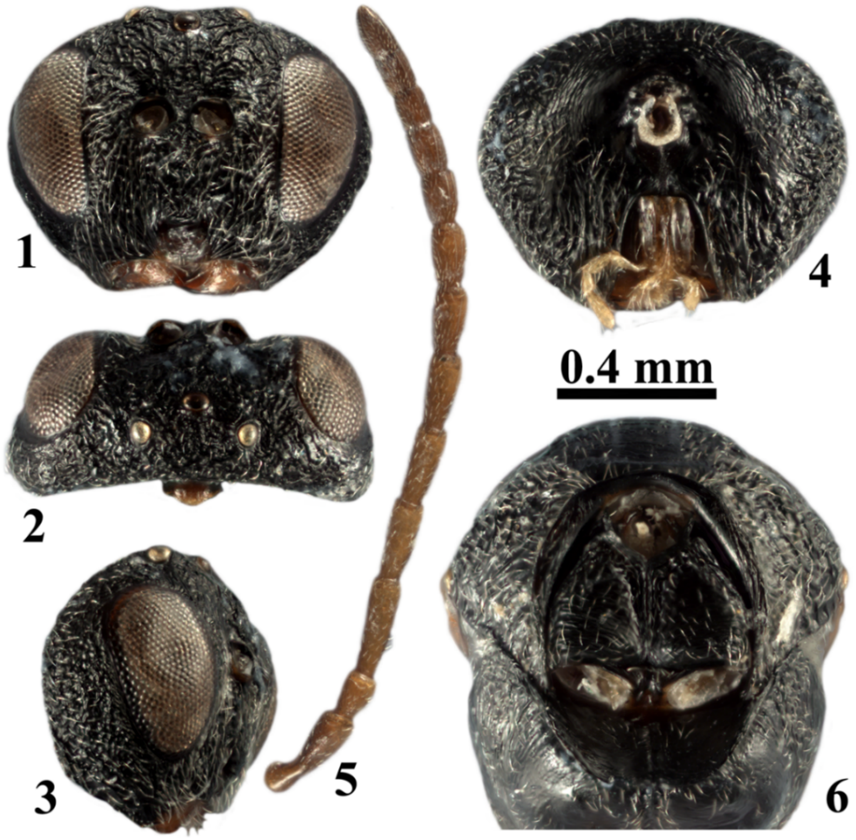
(Figs 1–22)

**Diagnosis:** Recently *Andricus cecconii* Kieffer, 1901 has been shown to include an undescribed sibling species (Tavakoli *et al*., 2019, 2021), *A. pseudocecconii*, herein described. The new species is most closely related phylogenetically to *A. cecconii,* the asexual generation of which is *A. megalucidus* Melika, Stone, Sadeghi & Pujade-Villar, 2004 (synonym in Shachar *et al*., 2018).

Galls of *A. pseudocecconii* most closely resemble the sexual generation galls of *A. cecconii* (Figs. 23–24, and see gall diagnosis below). They also somewhat resemble the sexual generation galls of *A. miriami* Shachar 2015 (*A. morula* (Shachar, Inbar & Dorchin, 2017 as a synonym)) from Israel, but *A. miriami* galls are much smaller and woodier, with a more elongate and mulberry-like (rather than spherical and walnut-kernel-like) structure (Fig. 25). Sexual generation galls of *A. miriami* are also covered with velvety pubescence and secrete nectar that attract insects (Shachar *et al*., 2015, 2017, 2018), whereas *A. pseudocecconii* galls are never sticky and lack velvety pubescence.

In *A. cecconii* antennae, legs and metasoma light brown to yellowish; head brown to dark brown, especially genae, gena distinctly narrower than transverse diameter of eye in lateral view; antennal torulus located slightly below mid height of eyes; wing venation light brown, projection of Rs+M reaching basalis in the upper half; pronotum without delicate parallel transverse striae laterally; metasoma longer than high in lateral view; in males mesopleuron without transverse striae while in *A. pseudocecconii* antennae, legs and metasoma dark brown to black; head is uniformly black, gena as broad as transverse diameter of eye in lateral view; antennal torulus located slightly above the mid height of eyes; wing venation light brown to yellow, projection of Rs+M reaching basalis at half its height; pronotum with delicate parallel transverse striae laterally; metasoma higher than long in lateral view; in males mesopleuron with delicate transverse striae.

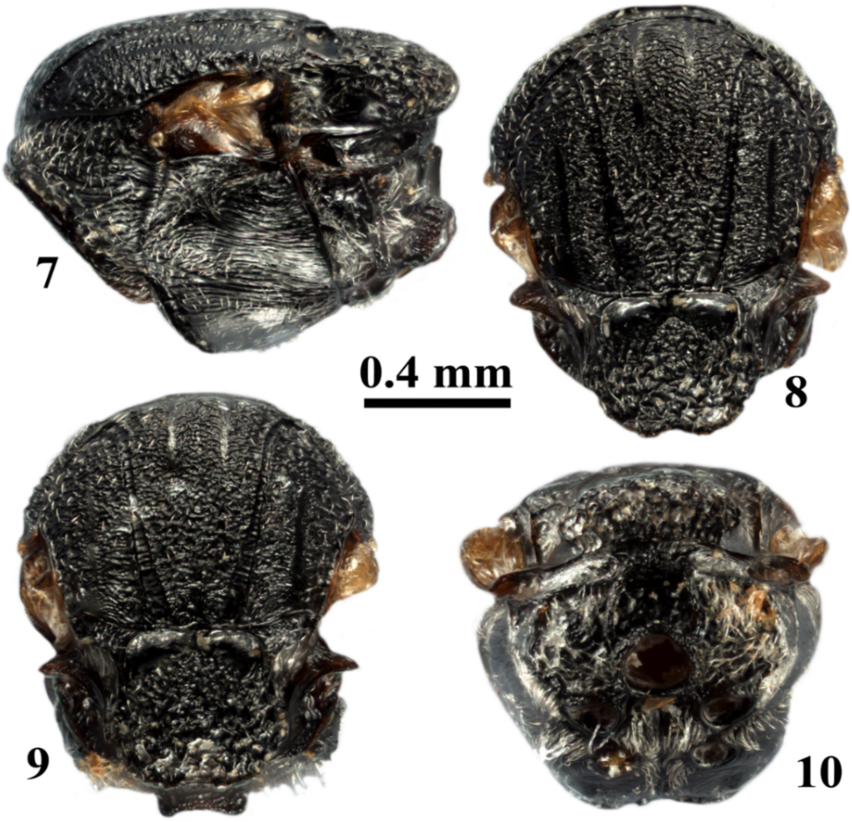
**Description**: Sexual female (Figs 1–10, 16, 18). Head, mesosoma black, antenna and legs dark brown; metasoma dark brown, dorsally darker than laterally.



**Figs 1–6.** *Andricus pseudococcconii* Melika, Tavakoli & Stone **sp. nov.**, sexual female: 1–4, head: 1, frontal view, 2, dorsal view, 3, lateral view, 4, posterior view. 5, antenna. 6, mesosoma and propleura, frontal view.

Head uniformly dull rugose, transverse, 1.3× as broad as high in frontal view, with sparse setae, denser on lower face, 2.0× as broad as long in dorsal view, as broad as mesosoma in frontal view. Gena dull rugose, slightly broadened behind eye in frontal view, as broad as transverse diameter of eye in lateral view. Malar space rugose, with striae radiating from clypeus and not reaching eye margin, malar sulcus absent; eye 3.0× as high as length of malar space. Inner margins of eyes slightly converging ventrally. POL 1.7× as long as OOL, OOL 2.4× as long as diameter of lateral ocellus and 1.4× as long as LOL, all ocelli ovate, of same size. Transfacial distance 1.2× as long as height of eye; torulus located slightly above mid height of eye, frons shorter than height of lower face, diameter of antennal torulus 3.5× as long as distance between them, distance between torulus and eye 1.2× as long as diameter of torulus; lower face dull rugose, with dense white setae and strong rugae radiating from clypeus and reaching to half the height of lower face; slightly elevated median area coriaceous, with a few setae. Clypeus slightly broader than high, impressed, alutaceous, glabrous, with delicate transverse striae, with a few long setae along ventral edge; ventrally not emarginate, without median incision; anterior tentorial pit rounded, distinct, epistomal sulcus distinct, clypeo-pleurostomal line well impressed. Frons with strong short irregular rugae, without striae and with few setae; area between toruli and torulus and eye rugose; interocellar area rugose. Vertex, occiput, postgena rugose, with scattered white long setae; postocciput smooth, glabrous, with concentric lines above occipital foramen; posterior tentorial pit large, elongate, area below impressed, glabrous; occipital foramen as high as height of smooth, glabrous postgenal bridge; hypostomal carina emarginate, continuing into postgenal sulcus; postgenal sulcus strongly bent outwards. Antenna slightly longer than head+mesosoma, with 11 flagellomeres, pedicel slightly longer than broad; F1 2.25× as long as pedicel and 1.3× as long as F2; F2=F3, F4 slightly longer than F5, F6=F7=F8, F9=F10=F11; placodeal sensilla on F5–F11.

Mesosoma 1.2× as long as high, with sparse white setae, except for dense setae on lateral propodeal area. Pronotum rugose, with sparse setae, with delicate parallel transverse striae laterally; propleuron delicately coriaceous, with strong transverse striae, with sparse short white setae. Mesoscutum uniformly dull rugose, with sparse white setae, slightly longer than broad (greatest width measured across mesoscutum at level of base of tegulae). Notaulus complete, distinctly impressed, bottom with transverse striae; anterior parallel line distinct, in the form of a bare, smooth stripe, extending to 1/5 length of mesoscutum; parapsidal line distinct, marked with broad impressed smooth glabrous stripes; median mesoscutal line short, with smooth glabrous bottom; parascutal carina narrow, reaching tegula. Mesoscutellum quadrangular, as long as broad; disk uniformly dull rugose, incised posteromedially and overhanging metanotum, with a few short white setae. Mesoscutellar foveae transverse, slightly broader than high, with smooth, glabrous bottom, separated by narrow elevated central carina. Mesopleuron with distinct transverse striae, with setae in anteroventral part; mesopleural triangle dull coriaceous, with numerous short white setae; dorsal and lateral axillar areas coriaceous, glabrous, with dense white setae; subaxillular bar smooth, glabrous, triangular, 2.0× as high as height of metanotal trough; metapleural sulcus reaching mesopleuron at half of its height, upper part of sulcus distinct, lower part of sulcus separating coriaceous area, with dense setae. Metascutellum coriaceous, as high as height of smooth, glabrous ventral impressed area; metanotal trough smooth, glabrous, with few setae; central propodeal area lyre-shaped, smooth, glabrous, with irregular interrupted rugae; lateral propodeal carinae strong, bent outwards in posterior 1/3; lateral propodeal area rugose, glabrous, with long dense white setae. Nucha short, glabrous, dorsally rugose, with sulci laterally. Tarsal claws toothed, with acute basal lobe.

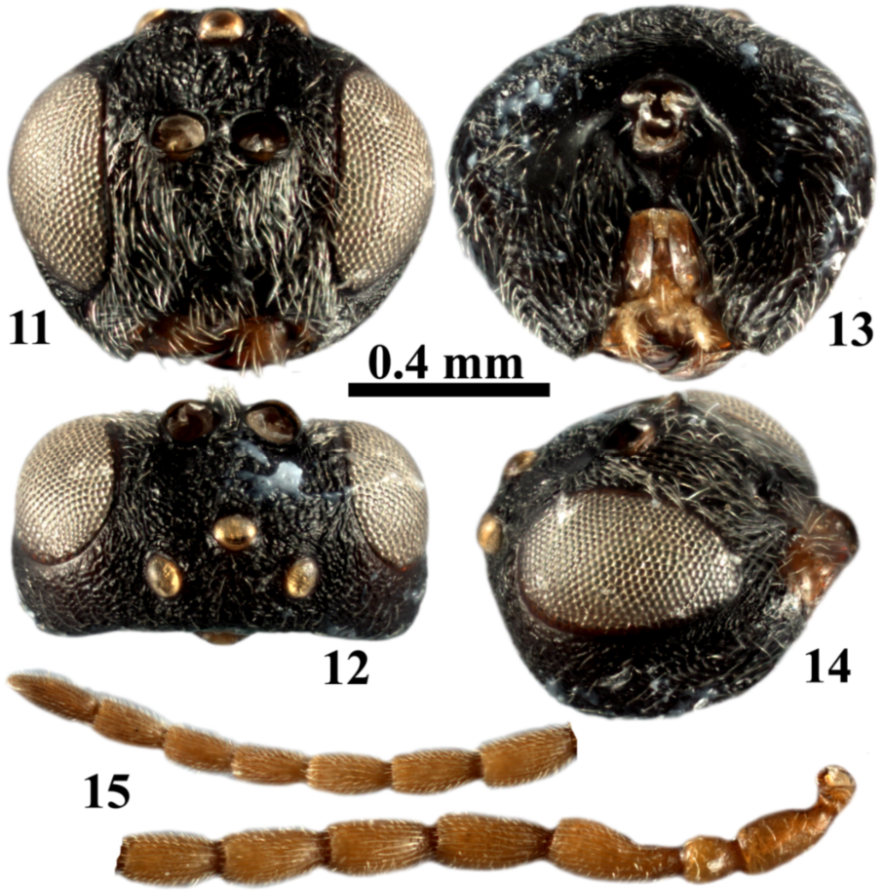


**Figs 7–10.** *Andricus pseudococcconii* Melika, Tavakoli & Stone **sp. nov.**, sexual female: 7, mesosoma, lateral view. 8–9, mesosoma, dorsal view. 10, metascutellum and propodeum, posterodorsal view.

Forewing longer than body, hyaline, with short cilia on margin, wing venation distinct, light brown, radial cell open, 3.8× as long as broad; R1 and Rs not reaching wing margin; areolet distinct, triangular, Rs+M light brown, visible for 3/4 of the distance between areolet and basalis, its projection reaching basalis at its half height.

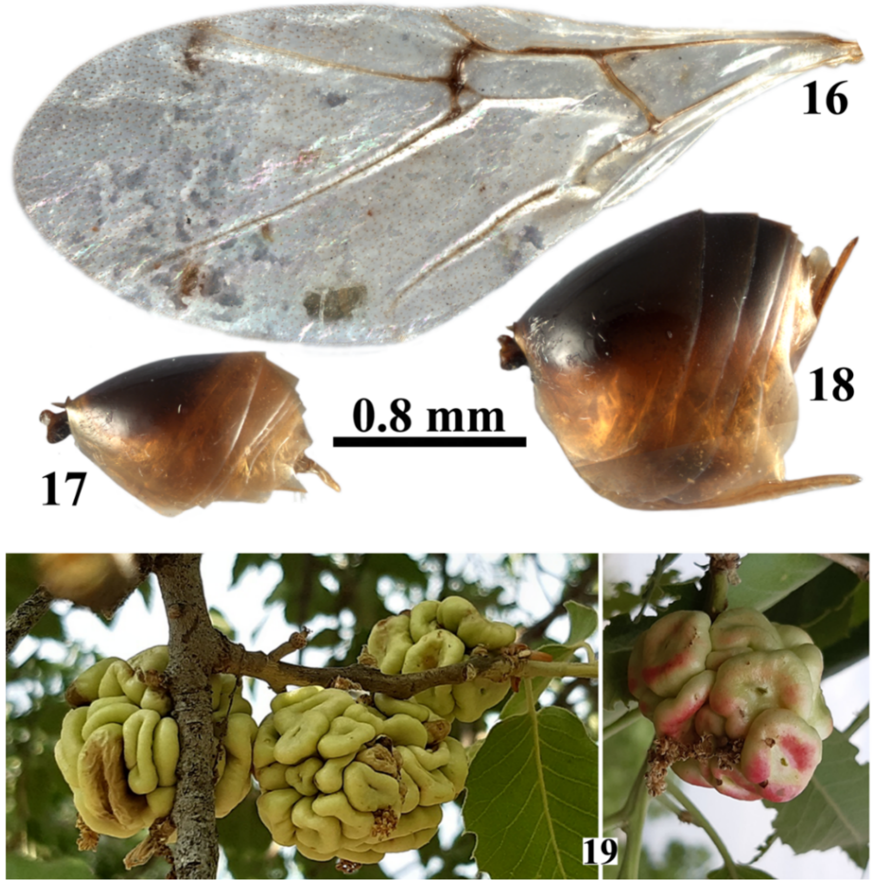
Metasoma slightly longer than head+mesosoma, higher than long in lateral view; 2nd metasomal tergite extending to half of metasoma length in dorsal view, with short white setae anterolaterally, without micropunctures; subsequent tergites and hypopygium smooth, glabrous, with indistinct micropunctures; prominent part of ventral spine of hypopygium 5.5× as long as broad in ventral view, with a few short setae ventrally. Body length 2.8–3.2 mm (n = 10).

Male (Figs 11–15, 17). Coloured as the female, except yellow-brown to yellow antenna and legs. Antenna slightly shorter than body, with 12 flagellomeres, F1 curved, excavated, broadened at the apex. Metasoma slightly shorter than head+mesosoma. Body length 2.6–3.1 mm (n = 10).



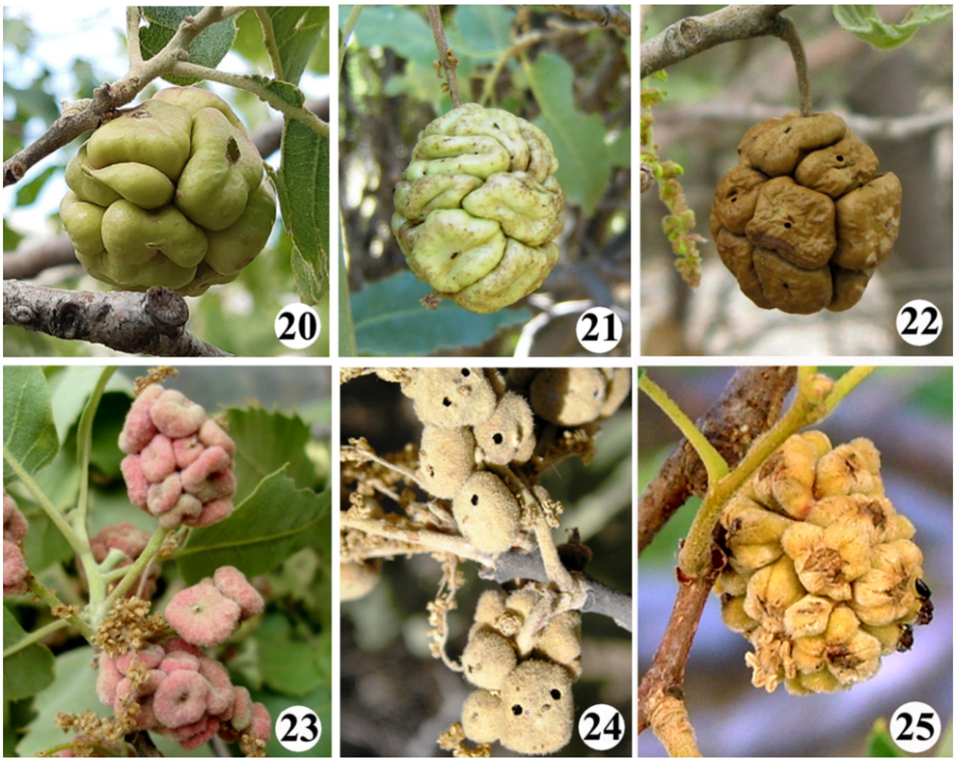
**Figs 11–15.** *Andricus pseudococecconii* Melika, Tavakoli & Stone **sp. nov.**, male: 11–14, head: 11, frontal view, 12, dorsal view, 13, posterior view, 14, lateral view. 15, antenna.

**Gall:** (Figs 19–22). The sexual generation galls of both *Andricus cecconii* and *A. pseudococecconii* develop on the catkins of *Quercus brantii* Lindl. (for which an old synonym is *Q. persica* Jaub. & Spach). In both species, galled catkins have a thickened axis and remain on the tree long after ungalled catkins have been shed. In both species, galls are commonly found in spherical to elongate aggregations of 10–18 (rarely 1-2) galls that can reach 25–30 mm in diameter and have a complex folded surface. In both species, each individual gall in the aggregation is irregularly conical, thin-walled, and contains 1–2 larval chambers. The outer face of individual mature galls reaches 5–10 mm in diameter, tapering to a narrow basal connection to the catkin axis. The galls of the two species can be found on the same trees, and differ as follows. In *A. cecconii*, the outer surface of each gall in the cluster resembles a Saturne peach fruit, with a velvety surface and a central depression. Initially, the gall is soft and green, becoming hard, woody and red to reddish brown or purple when mature. In contrast, the gall of *Andricus pseudococecconii* has a smooth (never velvety) surface and the aggregation commonly resembles a walnut kernel. Each gall in the cluster is pale yellow or yellowish brown when mature, and never reddish or purple.



**Figs 16–19.** *Andricus pseudococcconii* Melika, Tavakoli & Stone **sp. nov.** 16, forewing, female. 17–18, metasoma, lateral view: 17, male, 18, female. 19, gall.

**Biology:** Only the sexual generation is known, which induces galls on *Q. brantii* within *Quercus* section *Cerris* (Govaerts & Frodin, 1998; Denk *et al*., 2017). The gall begins development in early to mid May, and matures in late May, with adults emerging from late May. Sexual generation galls of *A. cecconii* in the same location begin their development at the same time, but mature later, with adults usually emerging from early to mid-July. Almost all Cynipini have cyclically parthenogenetic lifecycles, with strict alternation between sexual and parthenogenetic generations (Stone *et al*., 2002). Based on its phylogenetic proximity to *A. cecconii* and close similarity between these species in other aspects of their biology, we expect that the unknown asexual generation of *A. pseudocecconii* will induce galls on oaks in section *Quercus* - probably *Q. infectoria* - that closely resemble the large (to 50mm diameter) multilocular, spined, asexual generation of *A. cecconii*, previously known as *Andricus megalucidus* Melika, Stone, Sadeghi & Pujade-Villar, 2004 (synonymised in Shachar *et al*., 2018).



**Figs 20–25.** Galls, sexual generation: 20–22, *Andricus pseudocecconii* Melika, Tavakoli & Stone **sp. nov.** 23–24, *Andricus cecconii*. 25, *Andricus miriami* (*A. morula*, sexual gall as a synonym) (courtesy of N. Dorchin).

**Distribution:** Iran, Lorestan province (but see **Comments**, below).

**Type material:** Holotype female: "Iran, Lorestan, Gelaei, catkin gall, Code 11(2016), *Quercus brantii*, summer 2015, leg. M. Tavakoli 2015"; 7 females and 4 males with the same labels as the holotype; one female and 4 males "Iran, Lorestan, Gelaei, catkin gall (Code 16(2016), *Quercus brantii*, summer 2015, leg. M. Tavakoli 2015". The holotype female, 8 female and 8 male paratypes are deposited in PHDNRL, Budapest.

**Etymology:** Adult morphology, and sexual generation gall shape, structure and location are very similar to *Andricus cecconii* Kieffer, 1901. We have therefore named this species as "cecconii-like", hence *pseudocecconii*.

**Phylogenetic results:** Phylogenetic trees for both cytb and ITS2 show that the new species *A. pseudocecconii* is very closely related to *A. cecconii*, consistent with the previous confusion of the two taxa. The ITS2 data (Fig 26A) show individuals from the two species form reciprocally monophyletic groups, closely allied to both *A. miriami* and *A. lucidus*. Likewise, *A. pseudocecconii* and *A. cecconii* have discrete cytb haplotypes, although those of *A. cecconii* fall into two lineages (Fig 26B). The data from these two loci also confirms the matching of *A. megalucidus* as the asexual generation of *A. cecconii*, first demonstrated by Shachar *et al*. (2018).

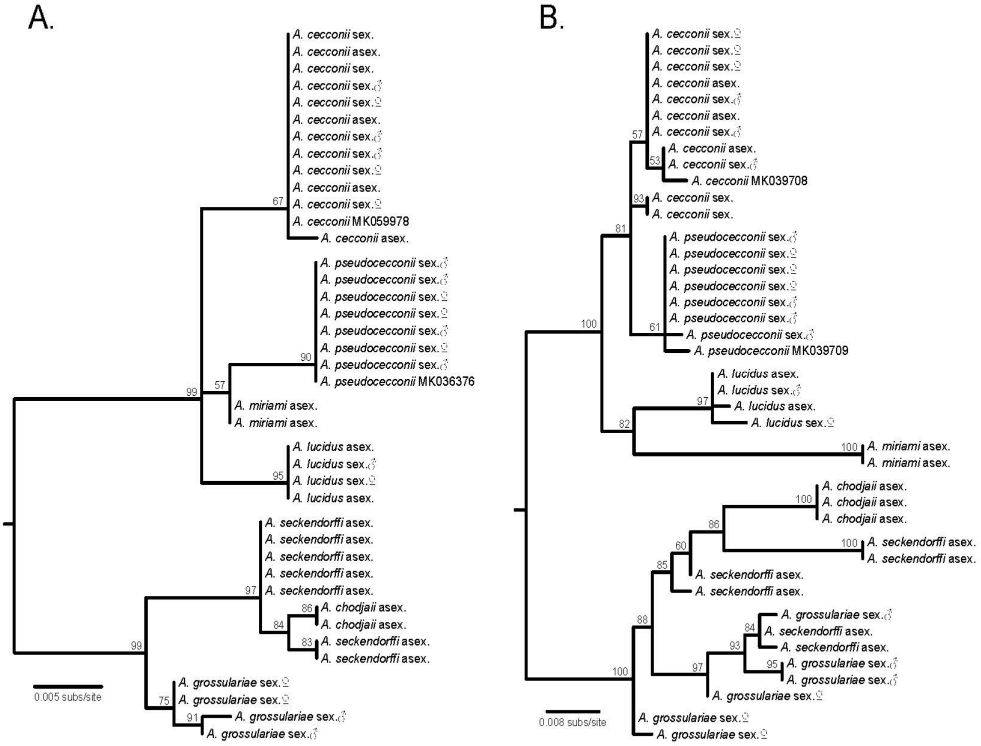


**Fig. 26.** Maximum likelihood trees containing *Andricus pseudocecconii* and close relatives: A. tree based on 496 base pairs of the nuclear ITS2 gene; B. tree based on 433 base pairs of the mitochondrial cytochrome *b* gene. The generation and gender of individuals is noted after the respective species name, as is the GenBank accession number for data taken from Tavakoli *et al.*, 2019; numbers above nodes show ultra-fast bootstrap support.

The reduced ability of cytb to resolve all individuals of a species into a single lineage is also highlighted by other species in this same group of *Andricus* species, with no pattern of reciprocally monophyletic clusters of cytb haplotypes among *A. grossulariae*, *A. seckendorffi* and *A. chodjaii*; however, these taxa form clear groupings in the nuclear ITS2 tree. This pattern is consistent with introgression of mitochondria across species boundaries among closely related species, a pattern previously observed in other clades of host-alternating *Andricus* (Nicholls *et al.*, 2012). This highlights the importance of using multiple genetic markers, including non-mitochondrial markers such as ITS2, when resolving the species status of oak gall wasps.

**Comments:** Sexual generation galls of *Andricus cecconii* are associated with *Quercus* section *Cerris* oaks: *Q. brantii* Lindl., *Q. ithaburensis* Decne, and *Q. libani* Olivier (and doubtfully with a section *Ilex* oak, *Q. coccifera* L.), and have been recorded (with or without the associated asexual generation gall, *A. megalucidus*) from Azerbaijan (Maisuradze, 1968), Greece (Pujade-Villar *et al.*, 2002), Israel (Sternlicht, 1968; Shachar *et al.*, 2018), Italy (Dalla Torre & Kieffer, 1910), Turkey (Azmaz & Katilmis, 2017) and Iran (Chodjai, 1980; Tavakoli *et al.*, 2021). The authors have collected this species also in Syria, Jordan, and Lebanon. Galls of the sexual generation develop from late February and adults emerge in April–July, depending on location. Galls of the asexual generation mature in autumn, and while most adults emerge from them in January, some emerge in early spring and some remain in diapause for at least 2 years. Given the high similarity between galls of *A. cecconii* and *A. pseudocecconii,* it is very likely that biological and distribution data previously attributed to *A cecconii* confounds information for both species.

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