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Ecological study of cynipidae galls on cork oak (*Quercus suber*) and zeen oak (*Quercus faginea*) in the Edough forest massif, Eastern Algeria

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Abstract: An investigation into the biodiversity of Cynipidae galls (Hymenoptera) residing within the oak forests of the Edough massif in Annaba (northeast Algeria) spanned from October 2016 to September 2019, aimed to update the faunal list of galls, assess their specialization and organ preferences, and evaluate the impact of seasonal fluctuations on their abundance. A total of 19783 galls were collected, belonging to 13 different species on both *Q. faginea* and *Q. suber*. All the oak Cynipidae galls collected belonged to eight genera: *Andricus*, *Synophrus*, *Callirhytis*, *Cynips*, *Cerroneuroterus*, *Neuroterus*, *Plagiotrochus* and *Pseudoneuroterus*. Abundance peaked during winter, contrasting with significantly lower levels in summer. The gall population exhibited a diverse and balanced distribution during spring (H=2.35; E=0.74). However, it was very low in summer (H=0.51; E=0.26). The results obtained thus are added to the available data concerning Cynipidae galls on oak trees (*Q. suber* and *Q. faginea*) in Algeria. This emphasizes the need for further research aimed at enhancing our comprehension of this specific population.

Key words: *Quercus*, Galle, Cynipidae, biodiversity, Algeria

Introduction

In Algeria, *Quercus* forests comprise a variety of oak species including cork oak (*Q. suber*), holm oak (*Q. ilex*), zeen oak *(Q. faginea*), afares oak (*Q. afares*), and kermes oak (*Q. coccifera*) (Boukreris et al., 2015). These forests contain galls that significantly enrich the entomological diversity of these ecosystems. Approximately half of the known galls in the Northern Hemisphere are found on trees within the Fagaceae family, particularly on *Quercus* (Dajoz, 1980). The emergence of these neoformations results from a deviation in the normal growth of the plant, impacting various parts such as leaves, peduncles, buds, catkins, as well as twigs, roots, and fruits (Dajoz, 1980). Galls arise on a variety of plants (Dauphin, 1993). The discussion surrounding their nature has persisted for over a century (Maresquelle & Meyer, 1965), yet our understanding of the causation and development of these neoformations remains limited (Jankiewicz et al., 2021). The Cynipinae (Hymenoptera: Cynipidae), specialized herbivorous insects, represent one of the largest insect families, boasting over 1400 known species worldwide and probably many more undescribed species. These Cynipidae are closely associated with oaks (Fagaceae: *Quercus*), each gall species being specific to an oak host (Nieves-Aldrey, 2001; Stone et al., 2009).

In addition, Cynipini have an unusual life cycle called heteronymy or cyclic parthenogenesis, characterised by the alternation between a gendered generation and an asexual generation that develop into two morphologically and temporally dissimilar galls, generally in different host tissues (Folliot, 1964; Pujade-Villar et al., 2001; Stone et al., 2002). This unusual life cycle means that each species of gall-forming cynipidae actually produces two ecologically and functionally independent organisms, creating a unique taxonomy (Brandão-Dias et al., 2022).

Monitoring of Cynipidae in the Mediterranean region is essential to protect forest health, especially oak trees, and preserve biodiversity. Infestations can weaken trees and alter ecosystems. Moreover, with climate change, the Mediterranean region is particularly vulnerable, and climatic variations can influence the distribution of cynids. Rigorous monitoring allows for the anticipation of environmental impacts and the development of effective conservation strategies to maintain the sustainability of forest ecosystems (Ali et al., 2022; Rodríguez et al., 2015).

In Algeria, as in most North African countries, studies on galls have so far been very limited. The most important contributions on Cynipidae galls have been made by Kieffer, 1897–1901; Marchal, 1897; Houard, 1914; Quezel, 1956; Benia et al., 2009; Pujade-Villar et al., 2010, 2012; Boukreris et al., 2015; Ghanema et al., 2016; Haddar et al., 2016; Adjami et al., 2017.

The aim of this study was to explore the diversity and abundance of the Cynipidae gall on cork oak (*Q.suber*) and zeen oak (*Q.faginea*) within the Edough forest massif in northeastern Algeria.

Material and methods

Study area

The study was carried out in the Edough massif (see Fig. 1), situated in the northeast of Algeria, which covering an area of 47,350 hectares and extends across the administrative territory of the Wilaya of Annaba. However, the forested area represents only 12,793 hectares, situated between latitudes 36°58' and 37°04' North and longitudes 7°17' and 7°47' East. The flora of the Edough forest is characterized by two main species: cork oak, covering an area of 3,419 hectares, and zeen oak, which thrives in the wettest areas such as the valley bottoms and the peaks (Oularbi & Zeghiche, 2009). Haut du formulaire.

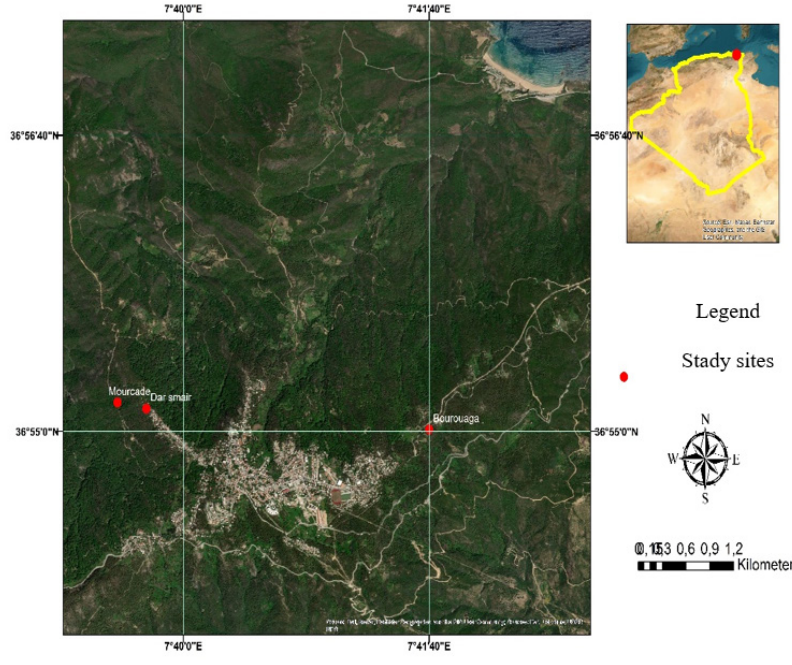


Fig. 1: Map and locations of the study sites in Edough northeastern Algeria (Source: ArcGIS 10.3).

The Edough benefits from a Mediterranean climate and is part of the humid bioclimatic belt. Regions with a Mediterranean climate are characterised by mild, wet winters and hot, dry summers (De Smet & Smith, 2001; Amira & Bougdah, 2018; Dong et al., 2021). Rainfall is very heavy (Mebarki, 2003) and the prevailing winds are characterised by very high speeds (Seltzer, 1946).

The selection of study sites was influenced by ecological factors, particularly the presence of oak trees and the abundance of galls. The study encompassed three forests: Bourouaga, Dar Smair, and Mourcade. These forests are located within the following Lambert coordinates **(**Tab. 1).

Tab. 1: Geographical details of the forests studied.

Forest names	**Geographical details**	**Oak species**
Bourouaga	36°91‘68,53“N 7°69‘44,34“E	*Q. suber*
Dar smair	36°55‘35,21“N 7°40‘52,23“E	*Q. suber et Q. faginea*
Mourcade	36°91‘93,85“N 7°65‘92,27“E	*Q. faginea*

Study method

For the collection of the galls, we adopted the method described by **Pujade-Villar et al., 2011,** as follows: Gals do not present any particular difficulty in being spotted on trees. It is enough to survey and observe carefully each part of the trees to detect and harvest many different shapes and sizes of galls. We also picked up the fallen gals. Gall collections were conducted in various oak forests (*Q. suber*, *Q. faginea* and mixed *Q. suber*, *Q. faginea*), at regular 4-week intervals over three consecutive years. The samples were placed in plastic bags, ensuring that galls collected from different host trees and those gathered from the ground were kept separate. Each bag was meticulously labeled with essential information such as the date of collection, locality, host species, etc. Upon collection, the galls were transported to the laboratory and sorted into separate tubes based on their morphotypes/species, where they awaited the emergence of adult insects. The galling insects were initially identified through our laboratory using the Guide of guide des galles de France et d'Europe (Dauphin, 2014) and subsequently verified at the University of Barcelona's laboratory in Spain.

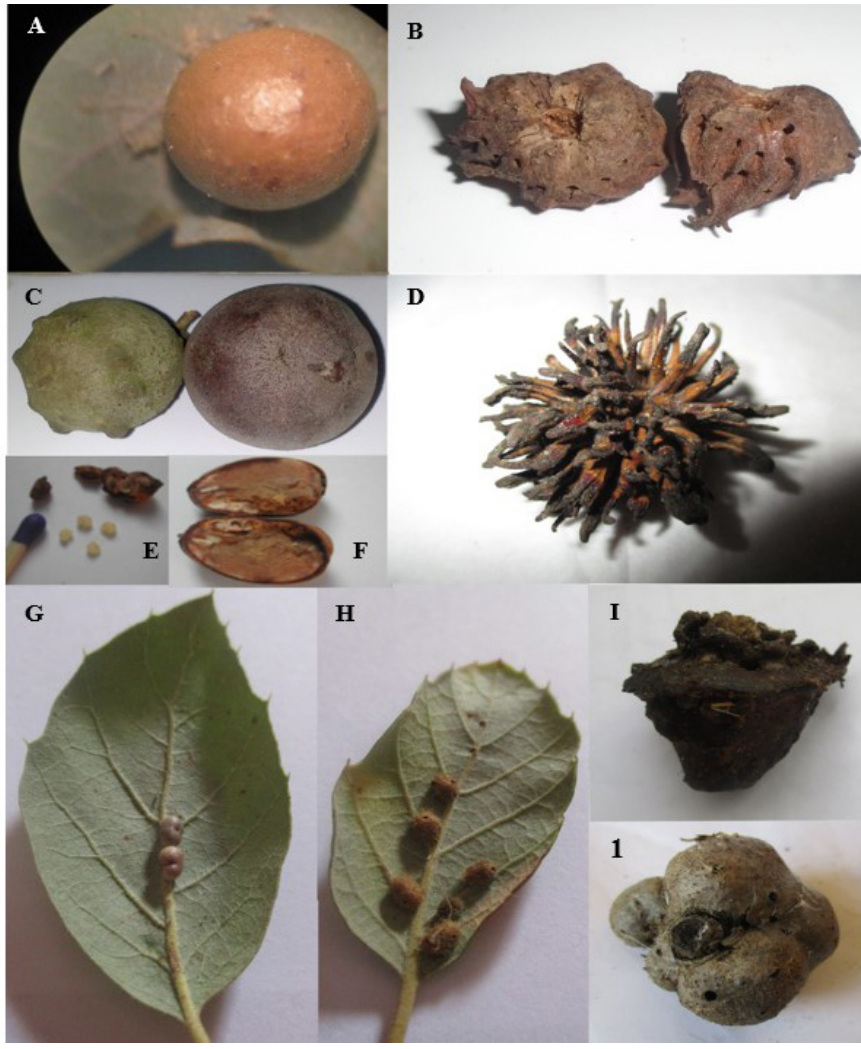


Fig. 2: Harvested gall plates. **A:** *C. quercus*; **B:** *A. coriarius*; **C:** *A. quercustozae*; **D:** *A. grossulariae*; **E-F:** *C. glandium*; **G:** *P. saliens*; **H:** *C. minutulus*; **I:** *A. pictus*; **J:** *S. olivieri* (Photos personnelles).

Data analyses and processing

The species richness and collected data were analyzed using numerical proportions of individuals and species. The Shannon-Weaver diversity index (H') and Equitability (E) of the galls were calculated based on the numerical abundance of the species using SPSS v23.0 software (IBM Corp., 2015). Correspondence Factor Analysis (CFA) was performed using ADE-4 software (Chessel & Doledec, 1992).

Results

Gall fauna

The identified species belong to the order Hymenoptera and the family Cynipidae, with 11 species attributed to the tribe Cynipini and two to the tribe Synergini. They are distributed across eight genera, with *Andricus* being the most prevalent with six species, followed by *Synophrus* with two species, and one species each for *Callirhytis, Cynips, Cerroneuroterus, Neuroterus, Plagiotrochus*, and *Pseudoneuroterus*. Each species exhibits high specificity to its host plant and target organ. Among these, only four species showed a gendered generation: *Andricus grossulariae, Neuroterus quercusbaccarum, Synophrus hispanicus*, and S. *olivieri*, while nine species displayed an asexual generation (Tab. 2).

Tab. 2: List of the identified galls on *Q. suber* et *Q. faginea* in the Edough massif.

Species	Generation	Host plant	Attacked organ
Andricus coriarius	asexual	*Q. faginea*	Buds
Andricus grossulariae	gendered	*Q. suber*	Chaton
Andricus grossulariae	asexual	*Q. faginea*	Buds
Andricus hispanicus	asexual	*Q. faginea*	Buds
Andricus pictus	asexual	*Q. faginea*	Acorns
Andricus quercustozae	asexual	*Q. faginea*	Buds
Callirhytis glandium	asexual	*Q. suber*	Acorns
Cynips quercus	asexual	*Q. faginea*	Leaves
Cerroneuroterus minutulus	asexual	*Q. suber*	Leaves
Neuroterus quercusbaccarum	gendered	*Q. faginea*	Leaves
Plagiotrochus amenti	asexual	*Q. suber*	branches
Pseudoneuroterus saliens	asexual	*Q. suber*	Leaves
Synophrus hispanicus	gendered	*Q. suber*	Branching
Synophrus olivieri	gendered	*Q. suber*	Branching

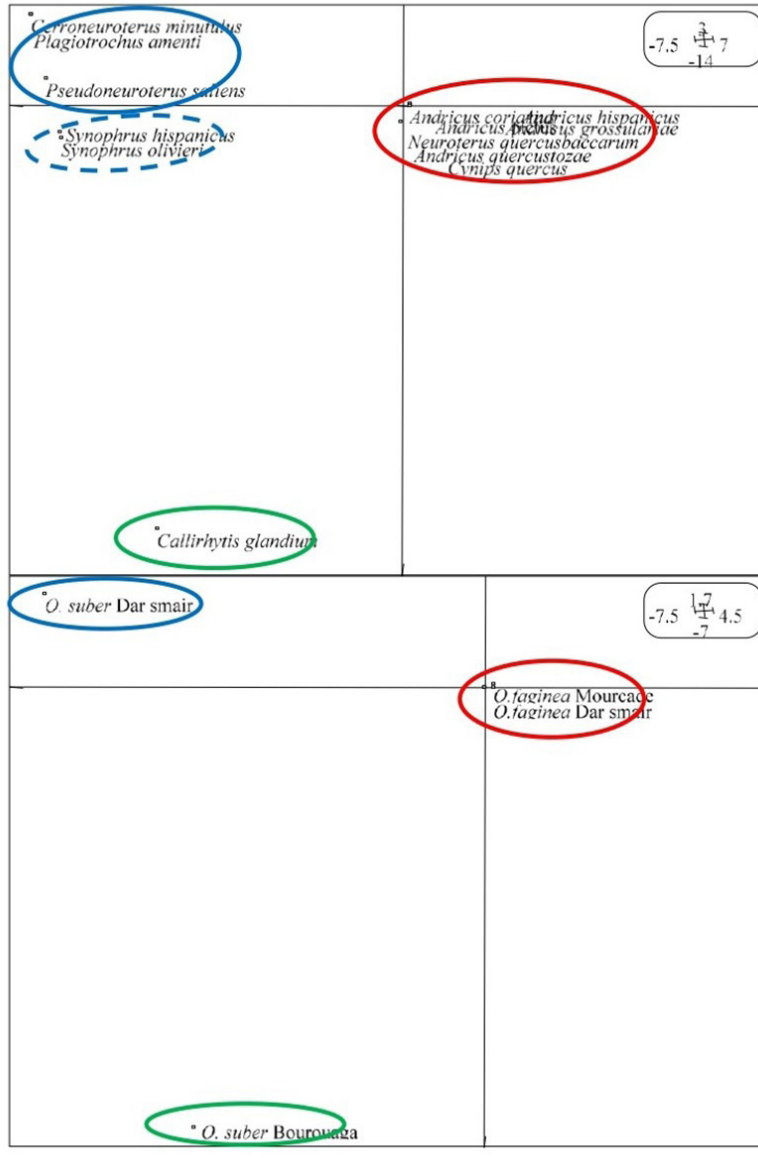
Analysis of the population structure

The distribution of galls in the sites

Multivariate statistical analysis using ADE-4 software (Chessel & Doledec, 1992) through Correspondence Factor Analysis (CFA) in its 1x2 graph, which encompasses 96% of the information, reveals a distinct differentiation between the two studied plant substrates: *Q. suber* and *Q. faginea*. This discrepancy is highlighted by factor 1 along of the ordinates

(Fig. 3). These two species are grouped together in the middle of the graph and are characterized by the presence of several species of galls.

On the other hand, the abscissa factor 2 contrasts the *Q. suber* of the Dar Smir region from those of *Q. faginea*. The first is dominated by three species (*P. amenti, C. minutulus* and *P. saliens*) and the second by a single *C. glandium*. Two other species of gall have also been recorded on the *Q. suber* of the two regions: *S. hispanicus* and *S. olivieri*.

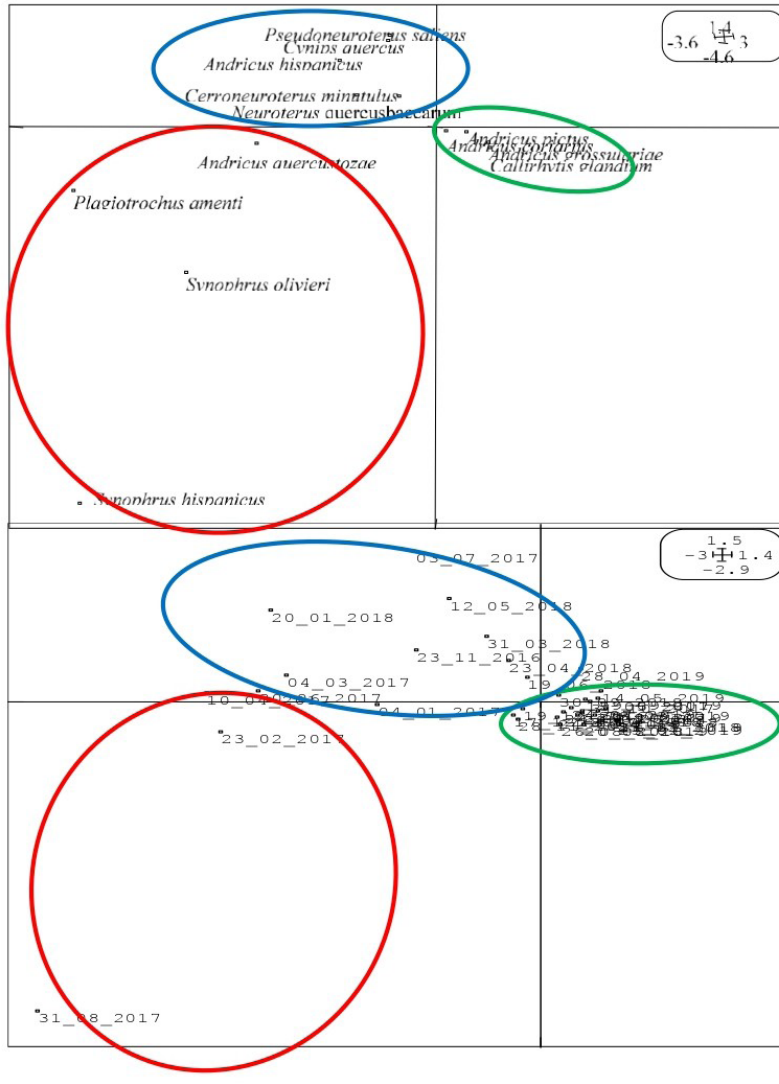


Graph 1: 1x2 factorial design of the AFC. Distribution of gall populations in the three sites. Axes of inertia: 0.74, 0.22, 0.03 and 0.01.

Manifestation of galls over time

In this 1x2 graph of the AFC, which holds 56%, we can see three main groups (Fig. 4), Group 1, which is opposed to the other two groups by the factor 1 of the ordinates (41%), includes the following species *A. pictus, C. glandium, A. grossulariae* and *A. coriarius* which are most common in February, October and November.

The second group comprises four species *S. hispanicus, S. olivieri, P. amenti* and *A. quercustozae*, which are most common in February and August. It is opposed to group 3 on axis 1 of the abscissa, which holds 15% of the information. This last group includes *N. quercusbaccarum, C. minutulus, A. hispanicus, P. saliens* and *C. quercus* are present almost all year round.



Graph 2: AFC 1x2 factorial plane. Manifestation of galls over time. Axes of inertia: 0.41, 0.15, 0.12 and 0.10.

Study of the ecological parameters

Abundance

In terms of abundance, which reflects the total number of individuals collected, galls are present throughout the year (refer to Fig. 5). The highest abundance was observed during the winter season, reaching a peak of 1,280 galls in February. Conversely, the months of June, July, and August were characterized by very low presence levels.

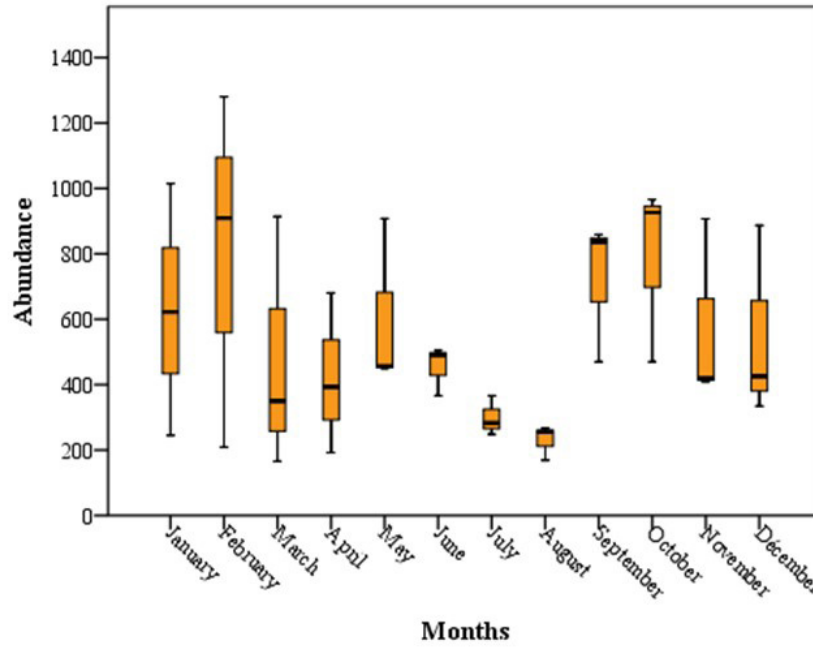


Fig. 3: Evolution in the number of harvested galls.

Specific richness

In terms of specific representativeness, which refers to the number of recorded species, the highest numbers were reported in November, with approximately ten species noted (see Fig. 6). This was followed by October and April. In contrast, the lowest values were observed in August, with only three species recorded.

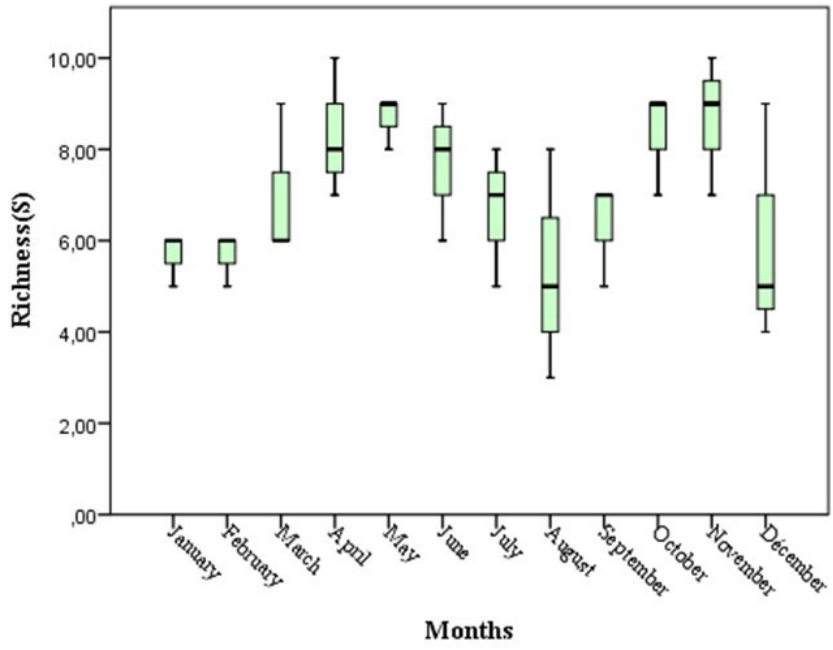


Fig. 4: Evolution Changes in the species richness of harvested galls.

Population balance indexes

The two graphs (Fig. 7, 8) for the two indexes show more or less similar trends, indicating that the greatest balances are observed during the month of May, proving that the individual representativeness of the species collected (2.35 for the H' index and 0.74 for the E index during 2018) is more or less similar. The lowest values were recorded during the month of August (H'=0.51 in August 2019) indicating that the population is dominated by a single *A. pictus* species. Note that during the month of May 2017 the equitability index recorded the lowest value (E=0.26 in May 2017) where the stand was made up of 8 species dominated by *A. pictus*.

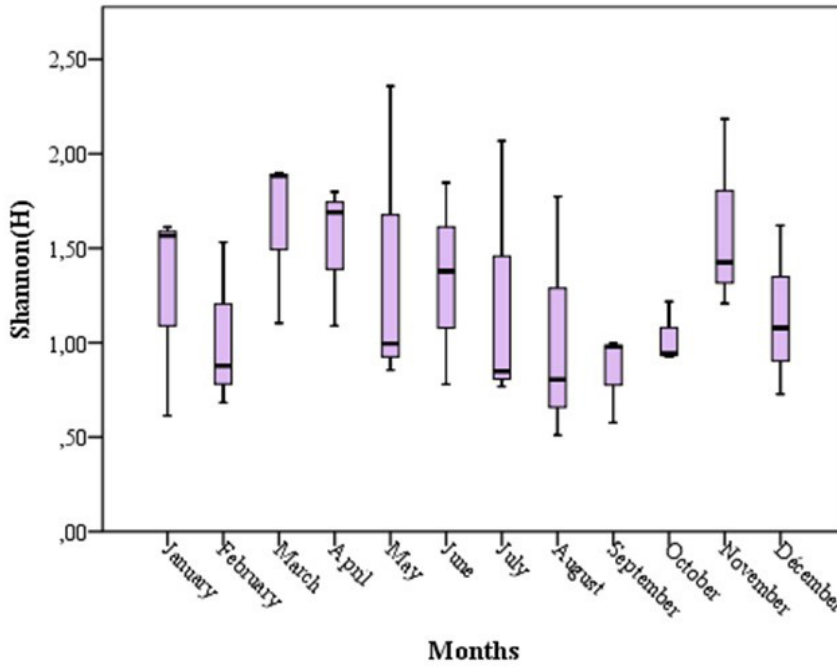


Fig. 5: Changes in the Shannon and Weaver specific diversity index (H).

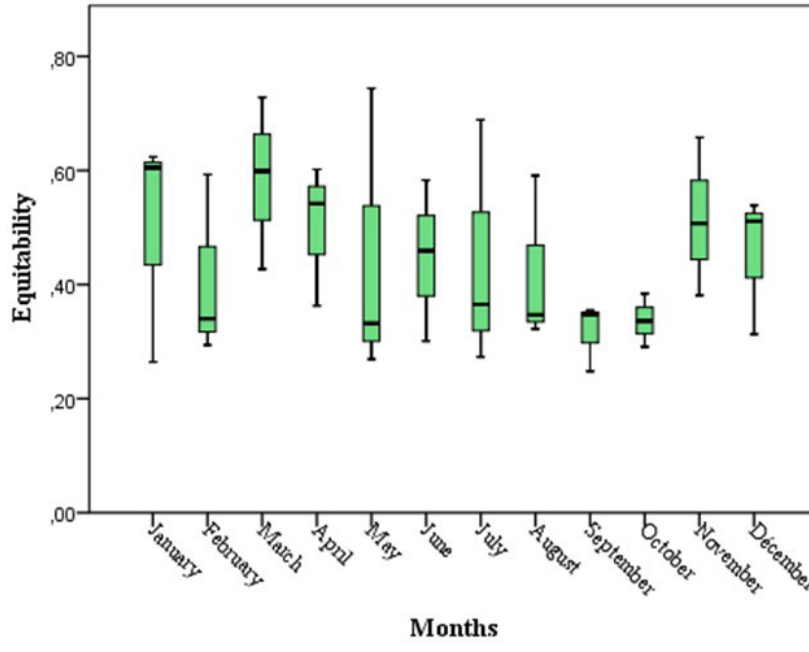


Fig. 6: Evolution of the equitability index (E) of the population of harvested galls.

Discussion

In North Africa, 86% of Cynipidae species grow on the genus *Quercus,* comprising 41 species that are particularly associated with *Q. suber, Q. faginea, Q. ilex, Q. coccifera, Q. canariensis,* and hybrid species such as *Q. afares and Q. kabylica* (Askew, 1984; Pujade-Villar et al., 2012).

In this study, we identified 13 species of Cynipidae galls developing on different parts of *Q. suber* et *Q. faginea*, dont *A. coriarius, A. grossulariae, A. pictus, A. hispanicus, A. quercustozae, C. glandium, C. quercus, C. minutulus, N. quercusbaccarum, P. amenti, P. saliens, S. Hispanicus* and *S. olivieri*. These species represent 38% of the gallicolous Cynipidae identified in Algeria, as repoted by Pujade-Villar et al., (2012). The absence of certain species can be attributed to the duration and timing of the study. According to Starzomski et al., (2008), the richness of oak trees is directly proportional to the richness of galls. In addition, some studies indicate that the local climate affects the distribution of specific gall species (Hosseinzadeh, 2012), so the collection effort is a crucial factor in studies on galls.

The collection of gall fauna suggests that asexual galls are more abundant, constituting 70% of the samples, while gendered galls account for only 30%. Conversely, research conducted by Ghanem et al. (2016) in eastern Algeria demonstrates a higher abundance of gendered species compared to asexual ones. According to Pujade -Villar (2012), most oak gall species are only known from asexual generation. Under certain environmental conditions, asexual reproduction can occur, resulting in the loss of the gendered generation (Pujade-Villar et al., 2001).

The results of our study show that each species of cynipidae is associated with one species of oak, previous studies have confirmed that each species of cynipidae is generally associated only with a closely related group of oak species (Stone et al, 2002; Melika et al., 2000).

In our study, we observed a distinct preference and specialization among the collected Cynipidae species for various organs of *Quercus* trees, encompassing leaves, acorns, catkins, twigs, and buds. On leaves, we identified 04 species, 02 species on *Q.suber* (*P. saliens* and *C. minutulus*) and the other two species *N. quercusbaccarum* and *C. quercus* on *Q. faginea*. In addition, 04 species on the buds of *Q. faginea (A. coriarius*, *A. hispanicus*, *A. quercustozae* and *A. grossulariae* (asexual), the gendered generation of the latter develops on the kittens of *Q. suber*. So, on the branches *P. amenti* and on twigs the presence of 2 species has been reported (*S. hispanicus* & *S. olivieri*). Indeed, *A. pictus*, *C. glandium* have been found on the acorns of *Q. faginea* and *Q. suber* correspondingly. This specialization has been confirmed by numerous studies (Weld, 1959; Abrahamson et al., 1998; Cook et al., 1998, 2002; Hayward et al., 2005, 2006; Pénzes et al., 2012; Leckey and Smith, 2015; Pascual-Alvarado et al., 2017; Ward, 2022). Studies have shown that environmental characteristics (Coutinho et al., 2019), physical and chemical changes of the plant (Höguland, 2014) and the quality of the host plant's tissues (Lawton & Strong, 1981; Costa, 2016) can influence the selection of target organs on *Quercus* hosts by Cynipidae. Consequently, gallicolous insects only attack target organs during the appropriate phenological stage of their specific plant (Raman, 2011).

Our results reveal a significant variation in gall abundance across seasons, with the highest levels observed in winter and the lowest in summer. These results align with previous studies conducted by Cronin et al. (2020) and López Núñez (2019). However, Chust et al., (2007) found no significant relationship between cynipid abundance and climatic factors such as rainfall and sunshine.

Furthermore, the galls typically develop during the spring season, reach maturity in summer, and the adult emerges the subsequent March (Stone 2002, Pujade-Villar et al., 2010).

Consequently, leaf galling was conducted during the autumn season (Abrahamson, 2003). In addition, the gendered generation develops during the spring (Pujade-Villar et al., 2001; Hood et al., 2018), while the asexual generation generally develops from summer to winter (Pujade-Villar et al., 2001). In our study, the cynipid population persists throughout the year, and an analysis of the organization of the gall population reveals that spring exhibits a notable diversity and balance compared to the other seasons (H'=2,35, E= 0,74). On the other hand, the lowest values were recorded in summer (H'=0.51, E=0.26). In addition, the number of species was highest in spring (10 species) and lowest in summer (3 species). Conversely, according to the findings of Hosseinzadeh (2012), summer and autumn are the seasons with the highest species richness. It is noted that the highest abundance of *Quercus* galls typically occurs during the season when resources are most abundant, which is autumn (Maldonado-López et al., 2016). The abundance of all cynipids was highly variable from one site to another, from one tree to another within the same site, and even from one part of the trees to another (Crawley & Akhteruzzaman, 1988). These galls tend to be more abundant in regions with intermediate latitudes, in arid and hot habitats, and featuring Mediterranean-type sclerophyllous vegetation. Moreover, diverse factors can influence the distribution patterns of galls, including abiotic factors, notably environmental conditions, spatial arrangement of trees, leaf characteristics, availability of bridge sites, and dispersal capacities (Meyer et al., 2020).

The findings presented herein contribute to the few data available concerning the abundance and species diversity of oak Cynipidae galls on *Q. suber* and *Q. fageniea* in Algeria.

Conclusion

The study revealed that 13 species of galls had been collected. On *Q. suber*, *A. grossulariae* (s), *C. glandium*, *C. minutulus*, *P. amenti*, *P. saliens*, *S. hispanicus* and *S. olivieri* were found. *A. coriarius*, *A. grossulariae* (asexual), *A. hispanicus*, *A. quercustozae*, *A. pictus*, *C. quercus* and *N. quercusbaccarum* settled on *Q. faginea*. This population was well diversified and balanced during the wet seasons, and very low during the dry seasons.

Additional monitoring and survey of galls is therefore necessary to identify new species, to assess the frequency of the various taxa in the massif, as well as their distribution and, above all, their bio ecology.

The specific diversity of cynipids and their wide host range shows that these hymenopterans play a fundamental role in maintaining the diversity of forest insect populations.

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