NEW CONTRIBUTION TO THE KNOWLEDGE OF MYCANGIA IN *PLATYPUS CYLINDRUS* (FABRICIUS, 1792), AND COMMENTS ABOUT THE VARIATION OF SOME MORPHOLOGICAL STRUCTURES IN MEDITERRANEAN ISOLATED POPULATIONS (COLEOPTERA: CURCULIONIDAE, PLATYPODINAE)

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Abstract: Ambrosia beetles have an obligate relationship with the ambrosia fungi that they feed on. Some of them have special structures called mycangia, used to convey fungi when they colonize new hosts. This paper describes the mycangia of the cork oak pinhole borer *Platypus cylindrus*, based on material collected in Algeria, France, Italy and Spain. The external morphology of these structures is compared with those of different species of the genus *Platypus*. The presence of non prothoracic mycangia is also explored and male genitalia variations are discussed.

Key words: Coleoptera, Curculionidae, Platypodinae, *Platypus cylindrus*, mycangia, morphology, Mediterranean basin, Algeria.

Introduction

All Platypodinae species are known as “ambrosia beetles”, they inhabit the sapwood of trees and both adults and larvae feed on ambrosia fungi carried and introduced by the adults inside the galleries dug on the wood (Beaver, 1989). Several fungi have already been isolated from *Platypus cylindrus* and its galleries in *Quercus* spp. (Baker, 1963; Cassier et al., 1996; Sousa et al., 1997; Morelet, 1998; Henriques et al., 2006, 2009; Belhoucine et al., 2011b, 2012; Belhoucine, 2013, among others); in Algeria only a study is reported Belhoucine et al. (2011b). The beetles and their fungi are intimately associated in a mutualistic relationship (Batra, 1963) causing damage of economic significance to trees and timber (Nakashima, 1975; Cassier et al., 1996). Besides the implication in insect feeding, ambrosia fungi might also be involved in other processes such as host weakness, through pathogenic action; decomposition of lignocellulolitic compounds, which helps gallery construction and enables fungi colonization; and/or antagonism that controls the growth of other fungi (Sousa & Ínácio, 2005; Henriques et al., 2006, 2009).

Beetles and their associated fungi have evolved morphological adaptations to ensure maintenance of symbiosis from generation to generation. The most obvious adaptations of the insects for consistent dispersal of certain fungi are specialized structures in the integument of the beetles used for the storage, transport and transmission of fungi (Kirisits, 2007).

These structures have been defined as mycangia or mycetangia (Batra, 1963; Francke-Grosman, 1967; Beaver, 1989; Berryman, 1989). Mycangia are cuticular tubes, pouches or pits of various sizes, associated with glandular cells that produce secretions which protect and preserve the spores of the associated fungi and opening to the surface of the body (Francke-Grosman, 1967; Batra, 1963; Beaver, 1989; Léveux et al., 1991; Six, 2003). Whitney (1982) and Six (2003) defined the mycangium as a structure that functions in the transport and protection of fungi, regardless whether glandular cells are present or not.

These structures are commonly classified on the basis of their location on the beetles and structural characteristics.
There is a great diversity in the location, form, structure and size of mycangia in xylomycetophagous species (Batra, 1963; Francke-Grosmann, 1967; Beaver, 1989; Berryman, 1989). Mycangia can be present on both sexes, only on the males or only on the females, depending on the species (Francke-Grosmann, 1967; Beaver, 1989). They may be found in many parts of the body (head, thorax, and elytra), but are usually constant in position in a particular genus (Beaver 1989). As yet, they have not been used in classification (Knížek & Beaver, 2007).

Within Platypodinae, only a few species have been described (Roche & Lhoste, 1960; Farris & Funk, 1965; Francke-Grosmann, 1966; Nakashima, 1971, 1972, 1975). In the genus Platypus, some species were used to describe the fungal transport organs (Wood, 1958; Nakashima, 1972, 1975; Atkinson, 1989, 2004; Moon et al., 2008; Kent, 2008). Most often, specialized structures of the integument for transporting fungi in this genus are located on the middle upper part of the prothorax (Nakashima, 1975) as a median disc of two to many mycangia (Kent, 2008). In some species, mycangia have been reported occurring as a single pouch at the rear of the preoral cavity, which is the case of female’s Cossotarsus niponicus Blandford (Nakashima, 1971, 1975). In the other hand, fungi were found in enlarged forecoxal and mesocoxal cavities in various male Platypus sp. (Nakashima, 1972, 1975).

The most important work on the mycangia’s structure of Platypus cylindrus was reported by Cassier et al. (1996) studying prothoracic mycangia using specimens from France and Portugal. However, no specimen from the North Africa was ever studied. Furthermore, some ambrosial species have coxal mycangia but according to our data these structures have never been examined in P. cylindrus. Primarily attacks the cork oak “Quercus suber” (Fagaceae)”, nevertheless other host are mentioned (Ferreira & Ferreira, 1989; Cassier et al., 1996; Cebeci & Ayberk, 2010). This species has originally a circummediterranean distribution but African and European populations (as well as those that are circumscribed in islands) are isolated from each other, so that could be signs of incipient speciation. The first record of P. cylindrus from Algeria was mentioned by Lucas (1849).

For all this mentioned, the aim of this study is: (i) to describe the thoracic mycangia of Platypus cylindrus collected in Algeria, not only to increase their morphological knowledge but also to discuss any differences from different populations, (ii) to compare the external morphology of mycangia in different species of Platypus, (iii) to explore the presence or absence of non prothoracic mycangia in P. cylindrus, and (iv) to examine whether some isolated populations from P. cylindrus could be considered or not morphologically different species.

Material and methods

Adult individuals of the ambrosia beetle Platypus cylindrus were collected from infested cork oak trees at the littoral forest of M’Sila, located 30 km west of Oran in the northwest region of Algeria. 80 beetles have been collected in 2002 (Bouhraoua) and 122 in 2010 (Belhoucine). These materials were maintained in alcohol 70% until their use. We have examined 97 males and 105 females from Algeria in this study. Also we have examined specimens from Cadiz and Girona (Spain), Nuoro (Sardinia, Italy) and Roquebrune sur Argens (France) (table I).

Electron microscope images were obtained with FEI Quanta 200 ESEM without any coating at 15 KV or with SEM Stereoscan Leica-360 with gold coating at 15 KV. The material was (i) treated according to standard procedures of these microscopes, (ii) untreated since the specimens were cleaned and the pictures have been made by Eva Prats (technician of “Serveis Cientifico-Tècnicos” at Barcelona University).

External optical images were obtained using a Carl Zeiss Tessovar Microcope and a Digital Camara PA Xcsm 3. Alcohol preserved specimens were dry out to natural conditions for the study. Images were adequate using the GIMP 2.8.2. Program.

Results

Thoracic mycangia

In Platypus sp. they are located only in dorsal prothorax area in both sexes (Fig.1), in the middle upper part. The mycangial plate is formed by pits (Fig.1c).

The intestinal pits are grouped into two areas on either side of a cuticular depression line corresponding to the longitudinal apoderme, localized in the median posterior pronotum forming two mycangium groups. The morphology of these structures shows a clear sexual dimorphism.

In females the pits are very numerous (380±50) (n= 72), usually with ovoid shape but we can observe some ones rounded or elongated (around 14 ± 3.5 x 11 ± 2.5 µm). They are distributed homogeneously on the two mycangial areas and cover a surface more or less circular, around (360±40) x (420±30) µm in average. This area is always incised dorsally and basally and they are more or less symmetrical along the median depression; these sub-unities, ovoid in shape, measure (360±40) x (190±20) µm. These mycangia are not very variable in shape (Fig. 2) in all specimens studied (n = 100).

In males the mycangial area is much reduced and the number of total pits is scarce. The pits are located on a small area about 150 x 140 µm and they have smaller dimensions (11±1.5) x (9±1) µm. The distribution of pits is not homogeneous even in males with the same number of pits and also it is different in both mycangial areas in the same specimen. The number is variable (Fig. 3); the maximum observed is 27 (n= 80) but usually varies between 5-15 (n = 70, 87.5% of studied population). Nevertheless, we have observed some specimens without thoracic mycangia (Fig. 3).

In both sexes, the openings pits are filled with different fungal spores (Fig. 1d, 1e). In some specimens (males and females), growing mycelia were observed at the entrance of the integumentary holes. Between the pits the cuticular surface is relatively smooth without gland openings neither setae. There is a spatial dissociation between the mycangial cavities and the sensorial setae (which are outside the mycangial area). The pits have not externally visible internal skeletal structure.

Coxal mycangia

Originally the structures observed in coxae I and II (Fig. 4) were suspected as potential mycangia pits, particularly in external view, however when the longitudinal cut was done into coxae it was observed that these “pits” correspond to
cuticular internal apodemes implantations which enforce the dorsal articulation of the coxae. It was probed that mycangial pits are not present in coxae of *Platypus cylindrus*. In other *Platypus* species, these structures are described (Nakashima, 1975).

**Male genitalia**

The differences observed in isolated populations of *P. cylindrus* led us to undertake a morphological study of the genitalia to visualize possible population differences and then to discuss the existence of more than one species of *P. cylindrus*.

The edeagus of this species is very simple, well sclerotized and tubular. The apical part is very small; the basal surface in the apical third presents a coarse and sparse punctuation. The genital segment is also very simple, in a "V" shape, without differentiating any particular structure.

In the studied specimens from M'Sila (Algeria) and the Natural Park of Alcornoquales (Cadiz, Spain) the curve of the

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Fig. 1. *Platypus cylindrus* female and male. a) adult beetle, b) dorsal prothorax, c) mycangial area, d) mycangial pits storing spores, e) details of a mycangial pit.
Fig. 2. Some variation shape of thoracic mycangia in Platypus cylindrus female from Algeria. Scale corresponds 100 µm. Fig. 3. Some variation in number and disposition of thoracic mycangia pits in Platypus cylindrus male from Algeria.

Table I. Origin and numbers of specimen studied.

<table>
<thead>
<tr>
<th>Origin Used in</th>
<th>Algeria (M’Sila)</th>
<th>Spain (Girona/Cadiz)</th>
<th>France (Roquebrune sur Argens)</th>
<th>Italy (Sardinia)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>♂</td>
<td>♀</td>
<td>♂</td>
<td>♀</td>
<td>♂</td>
</tr>
<tr>
<td>Mycangia</td>
<td>80</td>
<td>100</td>
<td>33</td>
<td>25</td>
<td>---</td>
</tr>
<tr>
<td>Coxal parts</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>---</td>
</tr>
<tr>
<td>Genital parts</td>
<td>12</td>
<td>---</td>
<td>23</td>
<td>---</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>105</td>
<td>63</td>
<td>32</td>
<td>7</td>
</tr>
</tbody>
</table>

The apical part is regularly rounded (Fig. 5a). In contrast to the specimens from the Montseny Natural Park (Girona, Spain), and also those from France and Italy, the curve of the apical part of the aedeagus is presented more or less incised in the middle (Fig. 5b). Also in these specimens the punctuation of parameral sheath is more dense and impressed, and the lateral projections in the basal part are longer.

For the observed differences apparently we have two populations, distinctly separated by small differences in the conformation of male copulatory organ, although these populations can not be distinguished by the external adults morphology. According to the studied specimens two different populations can be located: (i) in the south of the Iberian Peninsula and North Africa and (ii) in the peninsular area of Catalonia, France and Italy. These populations are confined to the areas occupied by Quercus suber (Fig. 5).

To differentiate these mentioned populations, several specimens from other areas colonized would be studied in the future in order to know their variability and the eventual intermediate transition stages between them.

Discussion

Some Scolytids and Platypodids carry mutualistic fungi in repositories in their integument (Francke-Grosman, 1963). Such organs may occur on the thorax, head or elytra of various species (Furniss et al., 1987) but also in the coxae of males in some species (Farris, 1963; Francke-Grosman, 1966, 1967; Nakashima, 1972, 1975). These are mycangia as called by Batra (1963). He described them as ectodermal origin, found mainly in females near glandular parts of the body. The morphology of the mycangia is usually genus specific (Beaver, 1989). However, variations can be found between species within the same genus (Nakashima, 1972, 1975).

In Platypodids, the genus Platypus includes around 120 species distributed in Europe and Africa to Japan and Australasia (Wood, 1993). Nevertheless, mycangia (Table I) have been rarely described in the Platypodinae (Nunberg, 1951; Lhoste & Roche, 1959, 1960; Farris & Funk, 1965; Nakashima, 1971, 1972, 1975; Cassier et al., 1996; Moon, 2008; Kent, 2008). The external morphology of Platypus mycangia is in general, ovoid in shape (Fig. 6) as in P. cylindrus. In some species, mycangia are only present in the female as P. quercivorus (Kinura, 2002) and P. jansoni (Nakashima 1975). In others, both sexes possess mycangia but reduced in some cases in the male (Table II, Fig. 6).

Several Platypus species have been introduced in Palaearctic area but only two species are considered as autochthonous: P. oxyurus and P. cylindrus. The first one is limited to
Fig. 4. Coxal apodemes in *P. cylindrus*.
the Pyrénées mountains, Corsica, Calabria, Greece and Turkey which is in accordance to Cassier et al. (1996) and it attacks only Abies alba (Balachowsky, 1949). However, P. cylindrus has a wide distribution throughout Eurasia and in the countries of the Mediterranean basin, including North Africa and it was introduced in Australia and New Zealand; it attacks the genus Quercus, Fagus, Castanea sativa and Prunus avium (Ferreira & Ferreira, 1989; Cassier et al., 1996; Cebezi & Ayberk, 2010). In Algeria, P. cylindrus has been observed only in Q. suber. The morphology of mycangia in both species from two sexes is different (Fig. 6). According to Cassier et al. (1996), the mycangia of P. cylindrus from Europe (Portugal and France) are similar to those from Algerian populations. Nevertheless, Algerian specimens have a mycangial area more or less circular incised dorsally and basally, but in Iberian specimens the mycangial region is usually wider than long and basally less incised.

The dimensions of the mycangial zone varied in European Platypus from 460 x 330 µm in the female to 140 x 140 µm for the male (Cassier et al., 1996). While in Algerian P. cylindrus, the mycangial area in females is about (360±40) x (410±30) µm against 150x140 µm in males. This is a dimorphic character in this genus and it has a defined shape (cordate, reniform, or like an arrow feather in outline) in different species. According to Nakashima (1975), Platypus genus is divided in five groups depending on mycetangia’s types; P. cylindrus is not included in Nakashima’ study but by all mentioned here it belongs to “Group B” because: (i) females has several tens or several hundred of integumentary pits-type mycetangia concentrated in the posterior part half of the pronotum, (ii) males mycetangia are similar to those of the female, but somewhat less developed or less numerous, and (iii) males have not coxal mycangia.

In Algerian females of P. cylindrus examined the number of pits in mycangia varied from 300 to 400, which is approximately similar to the European specimens (Cassier et al., 1996; Inacio et al., 2011). Even so, no female in our samples did show less than 300 pits while Cassier et al. (1996) found females with only 68 openings. In comparison with other species of the genus Platypus, P. cylindrus females have a double number of pits compared to female’s P. oxyurus with 150 holes only (Cassier et al., 1996); in addition P. cylindrus has more pits than P. calamus (around 250), P. calcicus (around 300) and P. (Shoreanus) bifurcus (between 250-270), according to Nakashima’s data (Nakashima, 1975). While P. Jansoni and P. Severini present more pits than P. cylindrus, with respectively 650 and 600, according to the same author. Farris & Funk (1965) reported 222 to 478 holes in P. wilsoni which is relatively similar to P. cylindrus.

In Algerian males’ P. cylindrus, the mycangia exhibited around 0-27 holes while the European specimens presented 5-25 (Cassier et al., 1996) with a maximum of 53 reported by Inacio et al. (2011), which is similar to some Asian Platypus (P. calamus, P. calicus, P. pseudocupulatus and P. severini) showing less than 20 openings in their mycangia (Nakashima, 1972, 1975). Some other species from Nakashima’s collection presented, by cons, more than 100 pits as P. curtus, P. (Shoreanus) bifurcus and P. (Shoreanus) multitalus. Several other males do not present mycangial holes as P. wilsoni (Farris & Funk, 1965), P. jansoni (Nakashima,1975) and P. quercivorus (Kinuura, 2002) (Table II). In this study we observed several P. cylindrus males without mycangial pits; except for Inacio et al. (2011) (Portugal) any other records in the bibliography referentiated the absence of mycangia in males of P. cylindrus in front the other European species P. oxyurus (Cassier et al., 1996).

The mycangia’s openings in the Algerian P. cylindrus under study, measured about (14 ± 3.5) x (11 ± 2.5) µm in females against (11±1,5) x (9±1) µm in males, which are slightly smaller then those in European specimens (Cassier et al., 1996). These data show also similarities with other Platypus species (Nakashima, 1972, 1975; Farris & Funk, 1965). But in some cases (Nakashima, 1975), the species present two kinds of mycangial openings as in female P. curtus (Fig. 6.7). Female P. quercivorus as reported by Kinuura (2002) presents only 5 to 10 sac-like organs located in the middle of the pronotum around the median sulcus (Fig. 6.9). These openings seem larger than those of P. cylindrus.

Fig. 5. Edeagus in ventral view of Platypus cylindrus: a) specimens from M’sila (Algeria); b) from Girona (Spain), and distribution map of the two different populations associated to aedeagus morphology. Scale = 0.2 mm. Map taken from http://www.euforgen.org/distribution_maps.html, February 2011.

Table II. Some species of Platypus with described mycangia according to Nakashima (1972, 1975), Cassier et al. (1996) & Kinuura (2002) data

<table>
<thead>
<tr>
<th>Platypus species with mycangia figured</th>
<th>Host</th>
<th>Distribution</th>
<th>Presence of thoracic mycangia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>females</td>
</tr>
<tr>
<td>P. calamus</td>
<td>Fagus crenata</td>
<td>Japan</td>
<td>+</td>
</tr>
<tr>
<td>P. calicus</td>
<td>Shorea sp2</td>
<td>Borneo</td>
<td>+</td>
</tr>
<tr>
<td>P. curtus</td>
<td>Shorea sp1</td>
<td>Filipinas</td>
<td>+</td>
</tr>
<tr>
<td>P. cylindrus</td>
<td>usually Quercus suber</td>
<td>Palaeartic occidental</td>
<td>+</td>
</tr>
<tr>
<td>P. jansoni</td>
<td>Camnos perma</td>
<td>Islas Salomon</td>
<td>+</td>
</tr>
<tr>
<td>P. oxyurus</td>
<td>Abies alba</td>
<td>Probably cirrimeditteranea</td>
<td>+</td>
</tr>
<tr>
<td>P. pseudocupulatus</td>
<td>Shorea sp2</td>
<td>Sumatra</td>
<td>+</td>
</tr>
<tr>
<td>P. quercivorus</td>
<td>Quercus quercivora</td>
<td>Japon</td>
<td>+</td>
</tr>
<tr>
<td>P. severini</td>
<td>Fagus crenata</td>
<td>Japan</td>
<td>+</td>
</tr>
<tr>
<td>(Shoreanus) bifurcus</td>
<td>Shorea sp1</td>
<td>Filipinas</td>
<td>+</td>
</tr>
<tr>
<td>(Shoreanus) mutilatus</td>
<td>Shorea sp2</td>
<td>Borneo</td>
<td>+</td>
</tr>
</tbody>
</table>

In Platipodids, according to different authors (Farris & Funk, 1965; Francke- Grosman, 1967; Nakasima, 1971), true mycangia have been found only in the female, while it is the male which attacks the host plant first. This is not the case of P. cylindrus, although the male possess mycangia, attacks first the tree host and actively begins boring the galleries (Bouhraoua, 2003; Sousa & Inacio, 2005; Bouhraoua & Villedamant, 2010; Belhoucine et al., 2011a; Belhoucine, 2013) creating a penetration corridor of about 1-2 cm in length starting from the bark wounds (Jover, 1952). The female continues the gallery construction started by the male (Husson, 1955). During this construction, the male role is restricted to sawdust removal (Hickin, 1963).

In males and females examined the openings were filled with fungal spores. In P. cylindrus both sexes carry in their mycangia relatively the same fungal species but the results obtained in a previous work (Belhoucine et al., 2011b) confirm the significant role played by females in the transport and inoculation of ectosymbiotic fungal spores, which agrees with others (Batra, 1963; Beaver, 1989). Nevertheless, we should not deny the role of male P. cylindrus in transporting fungi to be probably deposited on the gallery wall in its early stages of construction. But, this function has to be less important compared to that of females since in P. cylindrus: (i) males have limited number of pits compared to females and (ii) males without pits have been collected.

Fungi of Ophiostomatales order, represented by Ophiostoma quercus, O. stenoceras, O. nigrocarpum and Raffaelea montetyi, are the most important fungi carried by both sexes (Belhoucine et al., 2011b, 2012; Belhoucine, 2013). R. montetyi is identified as P. cylindrus primary ambrosia fungus in France (Morelet, 1998) and Portugal (Inacio et al., 2008, 2011; Henrique et al., 2009) and is probably the same brownish fungus described by Cassier et al. (1996). The other Ophiostoma species isolated are already known as important fungi to other bark beetles (Reay et al., 2002; Kirisits, 2007; Linnakoski et al., 2009), their importance to P. cylindrus must to be confirmed. Whereas, phylogenic stydies show that...
Ophiostoma and Raffaeleus species are closely related (Harrington et al., 2010). The isolation of Geosmithia longanii from P. cylindrus mycangia of both sexes is a new association for this beetle (Belhoucine et al., 2011b, 2012; Belhoucine, 2013). Members of Geosmithia genus have not been linked to Platypodinae until now thought they are usually part of fungal ambrosia beetle galleries (Kirschner, 2001; Kolarik et al., 2005).

The presence of growing mycelia at the entrance of the integumentary holes in both sexes of several specimens analysed agrees with observations of some authors as Whitney and Farris (1970) in some beetles and Inacio et al. (2011) in one specimen of P. cylindrus. Fungi, as reported in some studies, are present in mycangia in spore form and spraying cells (Farris, 1963) but also in yeast-like form (Harrington & Fraedrich, 2010; Yuceer et al., 2011). The mechanism by which mycangial fungi are maintained in the yeast-like form in the mycangia is not known but some authors suggested that chemical secretions from associated glandular cells surrounding mycangia may regulate fungal growth and morphology (Batra, 1963; Francke-Grossmann, 1967; Barras & Perry, 1971; Six, 2003; Beaver, 1989). In P. cylindrus, Cassier et al. (1996) described particular glandular cells in mycangium probably with this role.

Introduction of these fungi into the mycangia most likely occurs after adults emerge from pupal chambers (Whitney et al., 1987; kent, 2008). When ambrosia beetles emerge from parental hosts, they often carry mutualistic fungi within their mycangia (Barras, 1973; Hofstetter et al., 2006; Kent, 2008; Yuceer et al., 2011). Kent (2008) mentions this act from Austroplatypus incompertus, an Australian species; any data has been reported from P. cylindrus.

In some Scolytids and Platypodids, males have another type of mycangia represented by enlarged forecoxal and mesocoxal cavities in which many globular fungal materials or spores were observed (in Platypus severini, for example); however, in the same study (Nakashima, 1975) several species of Platypus did not show fungal materials although similar structures to those found in P. severini (as P. calicus, P. solidus, P. jansoni and P. calamus). In our study, the examination of coxal parts did not show any mycangia of this kind in P. cylindrus (Fig. 4); these pores observed are apodemas. It is possible that some of the pores previously reported in some Platypus spp. may carry fungal material only because of the shape of the structure and not because they may constitute a mycangial structure.

Conclusions

Platypus cylindrus is an ambrosia beetle whose prothoracic mycangia have a variable number of pores. In females, the shape of mycangial area is similar in different populations but instead the number of pites varies widely; in males, these pores are very scarce and very variably disposed. These differences are present not only in Algerian populations but also comparing European and Algerian populations. Females and males examined from Algeria have slightly less number of pores than European samples. Furthermore, coxal mycangia are absent in all populations examined.

Nevertheless, the study of genitalia does not allow separating species even though there are weak morphological differences between isolated populations. Then, for this moment, only one species is present attacking cork oak (Platypus cylindrus) considering these differences as a morphological gradation in genitalia. More material from different areas could be solving this aspect in a future.

Acknowledgements

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