The biology and physiological adaptation of mangrove ants to tidal flooding

MOGENS GISSEL NIELSEN

Aarhus University, Department of Biology, C.F. Møllers Alle 3, DK 8000 Aarhus C, Denmark

Corresponding author: mgn@stofanet.dk

ABSTRACT. Mangroves are an extreme biotope where ants are often described as the most abundant and influential group of insects. Yet very few studies have examined how ants survive the often extreme flooding of this biotope. This review summaries knowledge of the distribution and biology of mangrove ants, giving a particular insight into their adaptations, behavioral and physiological, and describes how some ant species survive during the extreme conditions with high temperatures, tidal flooding, very low oxygen and high carbon dioxide concentrations. Mangrove ants can be divided into three categories: 1) ants nesting in the aerial parts of the mangrove, 2) ants nesting in cavities inside trees or logs that are flooded periodically. 3) ants nesting in the mud within the tidal zone. The diversity of ants is lowest in categories 2 and 3, potentially because of the extreme environments they experience when submerged under water. Colonies of these ants can suffer extremely high temperatures, low oxygen and high CO_2 concentrations, with the responses of ants to these conditions differing between species. Workers of *Polyrhachis sokolova*, for example, survive in pockets of air with elevated CO_2 (up to 11%) when their mud nests become submerged and can swim across the waters surface if necessary. Soldiers of the twig nesting *Colobopsis anderseni* block nest entrances to prevent water influx, can survive normally lethally high nest temperatures and are the first ant known social insect to engage in anaerobic respiration when oxygen becomes limiting. Studies of the extreme habits of mangrove nesting ants provides insight into the adaptive capacity of ants.

INTRODUCTION

Mangroves are mainly distributed in the tropics and subtropics and comprise woody trees and shrubs growing in the tidal zone. The total area with mangroves is estimated to be 138 000 Km^2 (Giri et al. 2011), with approximately 1000 Km^2 a year being lost (Craft 2015, Richards et al. 2020) due to harvesting for wood, clearance for agriculture and other human development. Mangroves cover about 75 percent of all tropical coastlines (Saenger 2002), and line approximately 504 000 Km of coastline. Compared to other biomes, the diversity of mangrove tree species is low having only 55 species in 20 genera and 16 families worldwide (Hogarth 2007).

Mangroves are primarily regarded as marine ecosystems, but in most coastal mangroves the landward part will only be inundated at spring tides, whereas the outer part is flooded during each high tide. The productivity and biomass in mangrove ecosystem are high and greater than other aquatic ecosystems (Alongi 2002). Most terrestrial animals in the mangroves need to be protected from the seawater, and during high tide either escape up in the trees, move landward to dry areas or survive in tight air cavities under the surface.

Mangroves are also the breeding area for a vast number of blood-sucking insects, especially mosquitoes and midges, whose impact on humans has been the target for most of the insect studies carried out in these communities (Saenger 2002). Little has been published on other mangrove insects (Kathiresan and Bingham 2001, Yeo et al. 2021). Murphy (1990) and Lever (1952) describe how several insect species live in the intertidal floor of mangroves, retreating to sealed burrows or cocoons during high tide. They also describe some caterpillar that can survive submerged during high tide.

Ants show a remarkable ability to adapt to the majority of environmental conditions and they are present in nearly all biotopes except the high arctic and a few Islands. Their adaptability and social organization are the main contributor to the enormous global success and dominant influence in many ecosystems (Hölldobler and Wilson 1990). It should be no surprise, therefore, to find that ants are an important part of the mangrove fauna. However, most general information about the mangrove ant fauna derives from broader investigations and surveys, even though ants have been described as the most abundant and influential group of insects in these communities (Clay and Andersen 1996; Hogarth 2007; Caitano et al. 2018).

As there are few studies of these aspects of ant biology this review draws upon both published and unpublished records. Notably there is a great biogeographic bias in the information available because most investigations have been conducted around Darwin Harbor in Northern Australia (Nielsen 1997b, Nielsen et al. 2003, Nielsen et al. 2005, Nielsen et al. 2006, Nielsen and Christian 2007, Nielsen et al. 2009). Informa-

tion about mangrove ant faunas elsewhere is sparse and predominantly only rudimentary. Ants nesting in the mangrove is influenced differently by the tidal floodings, so we divide the mangrove ant fauna into three groups based on nesting: Group1 where ants nest in the trees above the tidal zone and are not influenced by the tide, Group 2 where ants nest in tree cavities in the tidal zone and are periodically inundated, and Group 3 where ants nest in the periodically inundated seabed.

Ant nests in the mangroves
Table 1. contains the ant species identified in the available mangrove literature.

Most ant species in mangroves are arboreal and are shared with neighboring forests (Hogarth 2007), but some of them have adapted most remarkably the intertidal habitat (Nielsen 2000).

Of those species that nest in the aerial parts of the plants, dead branches and decaying tree parts are the most common nesting locations (Cole 1980, Cole 1983a, b, Dejean et al. 2003, Wetterer 2018a, b).

On the Yucatan Peninsula, Mexico, the epiphytic plant *Aechmea bracteate* shelter small Myrmicinae in 31.3% of the ramets in peripheral cavities, and *Neoponera villosa* (Ponerinae) or *Dolichoderus bispinosus* (Dolichoderinae) in 91.9% of the central cavities of the ramets. Rotting, dry ramets shelter *Cyphomyrmex minutus*, a primitive Attini and numerous Ponerinae (Dejean and Olmsted 1997).

Nesting in epiphytic plants occurs also in the Australian mangroves where *Myrmicoda* plants host primarily *Iridomyrmex* species and *Hydnophodium formicarium* provides nest sites for several ant species (Huxley 1978).

Throughout northern Australia and southeast Asia through to India *Oecophylla smaragdina* is strictly arboreal and their nests are situated above the high-water level and not influenced by tides. Nevertheless, the ants have substantial impact on the mangrove fauna including other ant species. Studies carried out in a Thai mangrove has shown that the ants and their pheromones also could deter herbivorous insects and reduce crab herbivory (Offenberg et al. 2004a, Offenberg et al. 2004b, Offenberg et al. 2006a).

Table 1. No of ant species from investigations in mangroves.

	No. of species	No. of exotic species	No. of tidal nesting species	Mangrove general	Specific mangrove tree	Publication
New World						
Santa Carina Brazil	22			X		Lopes 1996
Kingston Jamaica	18	5	0		*Rhizophora mangle* Dead part of three	Wetterer 2018a
Florida USA	5		0		*Rhizophora mangle* Hollow twigs	Cole 1983a, b
Florida USA	18	8	0		*Rhizophora mangle* Dead part of three	Wetterer 2018b
Yucatan Peninsula Mexico	6		0		*Aechmea bracteate* Ramlets	Dejean and Olmsted 1997
Asia						
Shankou South China	9			X		Guofang 1997
Hong Kong	16			X		Fellowes 1999
Ca Mau Vietnam	15			X		Nielsen Unpublished
Ranong Thailand	12			X		Nielsen Unpublished
Singapore	37		3	X		Wang et al. 2020 Wang et al. 2018, Wang pers.com
Australia						
Darwin Harbor Australia	26		5	X		Clay and Andersen 1996 Nielsen 2000 Noske unpubl., Hofmann Unpubl.

A total of 37 ant species are known from Singapore mangroves, of which 25 were nesting arboreally, 4 species in epiphytes, 8 ground living and nesting in hollow branches and in the mud. There are only three tidal nesting ant species: *Pheidole sexspinosa, Odontomachus malignus* and *O. litoralis (*Wang et al., 2020) and they belong to groups 2 and 3. *Pheidole sexspinosa* has been found nesting inside a decayed stem of *Rhizophora sp.*, in cable roots of *Excoecaria agallocha,* and also in mangrove lobster mounds (Wang et al., 2018). *Odontomachus malignus* normally nests in coral rubble or coastal limestone, but nests can also be found in the seawards edge of mangrove (Olsen 2009, Wang et al., Wang per. com.). *Odontomachus litoralis* nests in mangrove lobster mounds (Wang per. com.).

Australia's mangroves covered approximately 11 000 Km^2 in 2010 and have contracted only slightly as a result of human activity (Lymburner et al. 2020). Darwin Harbor contains 36 of the 45 Australian mangrove tree species (Duke 2006). Darwin Harbor has very good conditions for developing an ideal mangrove. The amplitude of the tide is very large, being up to 8 meters at Spring tides. The mangrove is sheltered from large waves, and the coastline slopes very gently. The fringing mangroves of this region can be 500 to 700 m wide and are clearly divided into zones (Clay and Andersen 1996).

The ants are well known in these mangroves, and this location contains one of the greatest diversities of mangrove-adapted ant species known in the world.

Information on the influence of tides on the nesting behavior of mangrove ants is exclusively from Australia and Singapore. From these mangroves four different strategies have been found to avoid flooding of the nests as the tide comes in:

a) Tree-nesting ants that make their nests in large cavities in the trunk below the high water. These nests have the entrances below the nest chambers and ants survive in air pockets in the stems. *Polyrhachis terpsichore*, for example, makes nests in cavities in rotten parts of the stem, sealing holes or decaying parts of the bark around the nest above the nest entrance with a substance containing mud to make it airtight (Nielsen unpublished observations).

b) Soil-nesting ants. These ants can survive inundation in the mud if it contains a lot of clay or fine silt to make the substrate "waterproof". The nest structure has bell-shaped galleries that provide air pockets. This nest type is used by *O. malignus, P. sexspinosa, P. constricta,* and *P. sokolova.*

c) Ants using small cavities. These ants can use different kinds of cavities in the trees provided that the single narrow entrance is small enough for surface tension to prevent intrusion of the water as the tide comes in. The cavities can be self-made, natural or excavated by other insects. For example, *Crematogaster sp*. B uses this strategy, and nests specifically in *Avicennia marina*.

d) Head plugging. A special strategy, which is found in other species as a way of preventing entry of enemies, was found for the two species *Camponotus* sp. B and *Colobopsis anderseni,* which nest entirely in chambers in *Sonneratia alba* (Figure 1) twigs, where the founding queen has excavated a nest. During high tide they prevent water intrusion into the chambers by blocking the entrance with the head of a queen or soldier ant (Nielsen 2000).

Polyrhachis sokolova

This species is undoubtedly the most "marine" ant, nesting in the mangrove mud from the dryer *Ceriops tagal* to the wet *Rhizophora stylosa* zones amongst the tunnels of the crabs and mudskippers.

Fig. 1. *Sonneratia alba* fringe the outermost part of the mangrove in Darwin Harbor, Australia. Photo Dorthe Birkmose

Fig.2. Nest entrance of *Polyrhachis sokolova* covered with 30 cm seawater. Photo M. G. Nielsen

Fig. 3. Polyurethane-foam cast of a *Polyrhachis sokolova* nest with an intact root system. The lighter colored structures are the polyurethane filled galleries and the measuring tape indicate mud surface. The deepest part of the cast is 30 cm. Photo M. G. Nielsen

Fig. 4. *Polyrhachis sokolova* swimming during incoming tide, using the four front legs as oars and the two hind legs as rudders. Photo. A. Narendra

In Darwin Harbor most nests are situated at elevations from 7.2 to 6.0 meters above Lowest Astronomical Tide (LAT) which means that the nests at the upper range of the tide are immersed for 13% of the tidal cycle and for durations up to 1.25 hours. The nests at the bottom of the tidal range are covered by 64% of the cycle and the flooding can last up to 3.75 hours, with more than 2 meters of seawater above the nest (Fig. 2).

The nests are often polydomous with small volcano-like entrances made by the materials from the nest. In a study exploring the structure of the nests, polyurethane foam was injected into entrances until all the galleries were filled. Sections of the cast were removed after sawing the dense root-system and removing the mangrove mud (Nielsen 1997a). Fig. 3. shows the gallery cast and demonstrates how the galleries are most abundant in the upper 20 cm of the mud, but can reach down to 45 cm. The numerous small chambers and galleries are situated around the complex mangrove root systems which prevents the air-filled chambers from collapsing under the high pressure at high tide. The galleries are always excavated in materials which are quite air- and water-tight. Air caught in the convoluted gallery system will remain in small pockets, where the ants and brood can cluster to survive the flooding (Nielsen 1997a).

The flooding of the nests and the way in which the ants and brood retreat to the air pockets were captured in the film "Life in the Undergrowth" (Attenborough 2005).

Polyrhachis sokolova has adapted very successfully to its environment by developing an efficient swimming behavior, which is commonly used during foraging when crossing puddles or in and out coming tide (Fig. 4). The ants can walk and jump on the water surface like water striders. When swimming they break the surface with the legs, using the four front legs as oars and the two hind legs as rudders (Kohout 1988, Nielsen 1997a).

Crematogaster **sp. B**
This ant species is restricted to Grey Mangrove *Avicennia marina* where it nests in cavities in the branches in all parts of the tree. The ants are not capable of excavating the cavities themselves, instead being dependent on holes tunneled by

Table 2. Data of nests and nest content in two colonies of *Crematogaster sp. B* from *Avicennia marina* tree in the mangrove in Darwin Harbor.

A

	No of nests	Total volume of all nests ml (range in volume of nests)	No of workers	No of pupae	No of Larvae	No of alates	No of egg laying queens
Colony A	7	94.5 (3,00-32.3)	6130	820	3000	0	19
Colony B	12	252.2 (0.1-173.9)	6817	1637	12964	3	14

B

	Biomass of ants mg	No of coccids	Biomass of Coccids mg	Total biomass in nests mg	Mean biomass in mg per ml nest (range)
Colony A	3484	53	2625	6109	64.6 (10.9-190.7)
Colony B	5948	88	4150	10097	40.0 (14.9-239.8)

weevils (De Baar 1993). So the nest sites and the extension of the cavities are limited by the abundance of the beetles.

The nest structure of this species was examined by Nielsen (1997b) and (Nielsen unpublished) on *A. marina* trees in the outer part of the mangrove in Darwin Harbor, where the nests are inundated during most of the tides. The single tree investigated contained at least three separate colonies, and each colony consisted of several separate compartments/nests, each with an entrance less than two mm in diameter. The distribution of two colonies were identified and all compartments marked in the month of November. Each branch with a colony was removed from the tree and the branches were cut into pieces each containing one nest. In the laboratory, the branches were frozen and later split vertically in two parts. The length and volume of the nest was estimated and the contents sorted, counted, and the biomass determined. The data collected are presented in Table 2A and 2B:

In each colony the egg laying queens with numerous eggs were in one chamber, and larvae and pupae were found in most of the other chambers. The most conspicuous insect in many of the chambers were the large - up to 9 mm - long pink coccids, *Alecanopsis mirus*, which often occupied the whole surface of the cavity to which they were strongly attached as adults (Fig. 5). Only the first instar nymph is mobile and capable of moving around in the nest. The coccids feed on the plant's sap and produce honeydew, which is probably the main source of energy for the ants. The biomass of the coccids is a substantial part of the total biomass in the nest, and in one nest amounted to 240 mg per ml nest cavity (Table 2B) (Nielsen unpublished data).

In order to demonstrate that cavities in the tree were limiting the size of the colonies, two kinds of additional spaces were provided on an *A. marina* tree with a colony of *Crematogaster sp. B*. One type was an 8 mm hole in the branch of the mangrove tree with a stopper with a 2 mm hole. The second type of artificial nest was formed in the same way using a small branch from another tree and attached it to the tree with colony.

After 10 days, 7 nests were occupied by ants and three of them also contained first instar coccids (Nielsen unpublished data)

Colobopsis anderseni
Colobopsis anderseni (McArthur and Shattuck, 2001), - previously known as *Camponotus anderseni* - is restricted to *Sonneratia alba* trees and is the most common of the 10 ant species found on *S. alba* trees in Darwin Harbor (Nielsen 2000). *Sonneratia alba* trees dominate the vegetation zone at the lowest tidal levels, forming a belt ranging from 30 meters wide to single scattered trees. Founding *C. anderseni* queens gnaw holes in the young green terminal shoots of these trees, and afterwards carve out nest's cavities, which are later enlarged by daughter workers. Nest cavities extend through an entire internode (mean length 5.2 ± 0.6 cm), but almost never beyond (Fig. 6).

Mogens Gissel Nielsen

Fig. 5. Nest of *Crematogaster sp. B* in an *Avicennia marina* branch with the large pink coccids, *Alecanopsis mirus*. Photo Dorthe Birkmose

Fig. 6. The founding queen of *Colobopsis anderseni* in a newly excavated cavity in a twig of *Sonneratia alba.* Photo Dorthe Birkmose

The whole tree is colonised so that the nests in the upper parts are not inundated but the lowest nests, situated 5-6 m above LAT, are flooded for more than 60% of the time at high tide, with up to 2-3 meters of water above the nest. The founding of new nests commences when the tree reaches a height of 6-7 meters above LAT, and often all internodes in the new green shoots contain founding queens. Each tree functions as an island; consequently, the queens and workers have no contact with the neighboring trees.

The yearly growth of *S. alba* consists normally of three new green shoots from the terminal part of the twig, each containing 1-3 internodes. After a year the shoots turn brown and lignify. The founding queens are entirely restricted to the fresh green shoots for the excavation of new nest chambers. As the excavation continue and the twig grows thicker, the nest enlarges and all life stages can then be found in the cavity. In nest of *C. anderseni* adult ants, brood, and coccids can fill up to 50% of the cavity volume.

Both queens and large workers can effectively block the entrance holes with their heads when the branches are inundated, preventing seawater entering the nest but also stopping gas exchange (Fig. 7).

The workers *C. anderseni* are seldom seen outside on the *Sonneratia* branches and apparently rely almost entirely on carbohydrate secretions and predation on the single species of a large (up to 8 mm long) undescribed *Myzolecanium* coccid, which is found only in *C. anderseni* nests (Nielsen 2000). Apparently, these coccids can disperse independently, possibly by the wind, as well as being transported by worker ants (P. Gullan pers.com.). They are found in almost every inhabited nest chamber (mean number 3.9, range 1-16, N = 225). Two other undescribed coccid species are found in the nests of the other ant species in *S. alba* trees.

Fig. 7. A *Colobopsis anderseni* soldier blocks the nest entrance to prevent water intrusion. Photo M. G. Nielsen.

Fig. 8. An opened nest of *Colobopsis anderseni.* Adult ants, brood, and coccids can fill up to 50% of the cavity volume. Photo M. G. Nielsen.

Surveys of more than 600 nests have shown that queens in terminal shoots tend to disappear within a year, until just one old queen, in the first formed chamber at the base of the branch, remains (Nielsen and Birkmose, unpublished data). At that stage the colony consists of only one nest with an egg laying queen and up to 60 nests with just brood, workers, and coccids, but the excavation of each nest was commenced by a colonizing queen. In the 225 nests whose contents were counted, there was on average (with standard error) 15.9 ± 14.2 workers, 3.4 ± 2.5 larvae, 2.5 ± 2.4 pupae, 1.5 ± 1.4 alates (Nielsen 2000).

Nielsen (unpublished data) recorded the temperature (Tinytag datalogger) in the first 16 days in April in the nests of *C. anderseni* and found that the mean daily maximum temperature $\pm$SD inside the nests was 40.1 ± 1.6 $^{\circ}$C which was 4.6 ± 1.5 $^{\circ}$C warmer than the air temperature. The highest temperature measured was 43 $^{\circ}$C, which is close to lethal temperature for insects, and the air temperature was only 35 $^{\circ}$C. The air temperature at the nest site in the mangrove was 2.5 $^{\circ}$C warmer than the official temperature for Darwin (Nielsen unpublished data).

When the branches grow and the thickness of the walls in the nest increase, and when the wall reaches more than 10 to 12 mm the coccids cannot reach the phloem and they die. So, to maintain the colony's food supply, there is a demand for new nest sites with thin nest walls. However, the old queen is confined in her nest because of her swollen gaster and is therefore unable to gnaw holes for new nest sites and the workers are unable to gnaw a hole for themselves. Consequently, the colony will die out if they don't recruit new queens to excavate new thin-walled nests. Vacated nest cavities can be used by other ant species such as species of *Crematogaster*, *Tetraponera punctulata* and *Tapinoma* spp.

To investigate further why the newly settled queens disappear microsatellite techniques were used to analyse the genetic structure of the colony of nests (Nielsen, Pedersen and Boomsma unpublished data).

Maps of all the nets in four branches were constructed, and from all nests DNA were extracted from workers, sexuals and brood. Three microsatellite loci were used to analyze the genetic structure. (More detailed description of methods in Appendix 2)

The results show clearly, that the workers and brood in the nests with missing queens were genetically different from those in the nest with the remaining dominant queen. It is thought that the missing queens were killed by the workers produced by the dominant queen and the orphaned workers and brood were adopted by the nest with the dominant queen, even though they mostly remained in the home nest. The micro satellite investigation confirmed this but did also identify that a few had moved to other parts of the colony.

Thus, it seems that a colony get new workers from new queens with coccids enabling the founding nest, with the dominant queen, to survive for her full life span. When the old queen does die, she may be replaced by one of the newly established queens on the branch.

Nest gas concentrations and physiological adaptations

The mangrove ants that are subjected to regular flooding by tidal cycles are living in a stressful environment. Not only are they often subjected to high temperatures but, for varying lengths of time, there is no opportunity for gas exchange with the atmosphere. Ants can be tolerant of high temperatures (Bujan *et al.* 2020) and have adapted to conditions associated with colonial life such as elevated levels of CO_2 concentration. Fungus growing ants have often highly elevated CO_2 concentration in their nests. Romer et al. (2018) and Kleineidam and Roces (2000) investigated the carbon dioxide concentration in the giant nests of the leaf cutting ant *Atta vollenweideri*, and found that the carbon dioxide concentration never exceeded 2.8% in the dump area below the fungus garden, and the highest concentration in the nesting area was 1.5%. Sousa et al. (2021) measured the CO_2 concentration in the nests of *Atta sexdens* and found values up to 5.7 %, whereas the harvester ant *Pogonomyrmex badius* had concentration 0.2% CO_2. The CO_2 concentration in ant nests in the ground are strongly influenced by the CO_2 concentration in the soil atmosphere, which normally range from 0.3 to 3.0 %, but can exceed 10 % in anaerobic soils (Lavelle and Spain 2001). The atmospheric concentration is 0.04 %, and even large nests of *O. smaragdina* have only slightly elevated CO_2 concentration.
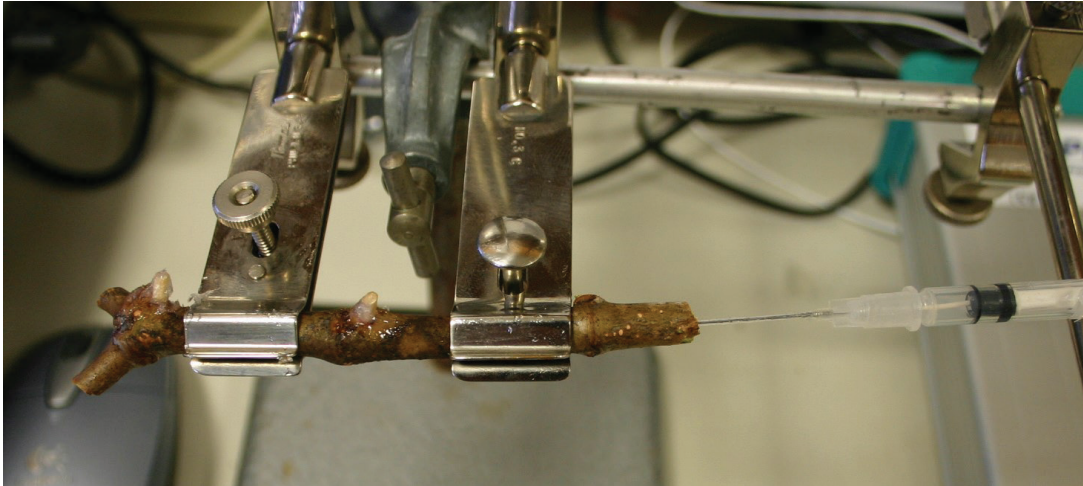
Fig. 9. A piece of a *Sonneratia alba* twig with a nest of *Colobopsis anderseni* in the laboratory. The nest entrance is blocked with a wooden stopper and glue, to simulate inundated conditions. A fiber-optic micro oxygen sensor is inserted through a hypodermic needle 2-5 mm into the nest cavity.
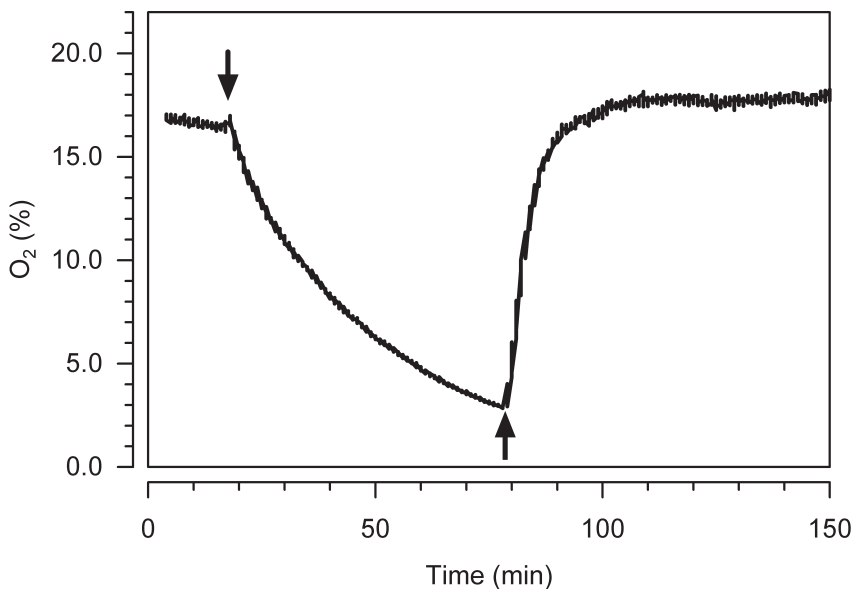
Fig. 10. O_2 depression during and after a simulated inundation of the nest of *Colobopsis anderseni*. The arrows indicate the nest closing and opening. Data from Nielsen et al. (2009).

During high tide *P. sokolova* retreats to air pockets in the nest until the water level in the nest drains. During this period the ants and brood only have a limited amount of O_2 available and must cope with the increasing concentrations of CO_2. Measurements of the CO_2 concentration in air samples from nest chambers at different depth shows great variation, from 2% to 11% CO2 (Nielsen et al. 2003). By comparing the CO_2 con-

centration from artificial galleries without ants, and the respiration of ants and mud samples, we could estimate that the ants only contributed 10 to 15% of total CO_2 concentration and the remaining from the microbiological respiration in the mud.

The strategy of *C. anderseni* is quite different to that of *P. sokolova* and more like that of other arboreal ants in the tidal zone and easier to investigate. Each small nest chamber in *C. ander-*

seni colonies is separate from the other chambers and there is only one entrance. Shortly after the excavation, the twig will get woody and all gas exchange takes place through the entrance.

The CO_2 concentration in the nest of *C. anderseni* were measured in the laboratory from gas samples from the nests in the field. The mean $\pm$SD concentration of CO_2 in the nests was 5.5 ± 3.2 % (range 1.5 –12.5%). The very large deviations due to the variations in the content of biomass, where the mean $\pm$ SD biomass volume in the nests was 22.7 ± 9.4 % of the total nest volume (range 1.5 to 49.6 %) (Nielsen et al. 2006).

The production of CO_2 in whole sealed nests in periods from 0.5 to 240 minutes were measured and the respiratory rates (uL CO_2 per mg per hour) were calculated at the end of the experiments together with the concentration of CO_2. The CO_2 concentration reached extremely high levels, over 30%. At 10 % and 25 % CO_2 the respiratory rate had decreased to 18,9 % and 1.8 % of rates in atmospheric air, respectably (Nielsen et al. 2006). (More detailed description of methods in Appendix 3)

Hypoxic conditions and oxygen supply in nests of the mangrove ant, *C. anderseni*, during and after inundation were investigated under semi-natural conditions (Nielsen et al. 2009). A hypodermic needle, with a fiber-optic micro sensor connected to an oxygen meter (PreSens Microx TX3) was inserted into the nest, such that the sensor tip extended 2–5 mm inside the cavity (Fig. 9). The O_2 concentrations inside the nests were measured continuously, and when stabilized, the nest entrance was then sealed. The seals were removed after 1–2 hours when the O_2 concentration had fallen to 4% or less and measurements continued until the values returned to the starting level (Fig. 10).

During normal conditions with open nests, the oxygen level is substantially lower in the parts at greatest distance from the entrance, such that in a 120 mm long nest the oxygen concentration in the air can be as low as 15.7%. During simulated inundation described above, the oxygen concentration dropped to very low levels, sometimes less than 0.5% after one hour. After opening the nest entrance, the oxygen level was restored to the starting level in about 20 minutes in a 100 mm long nest.
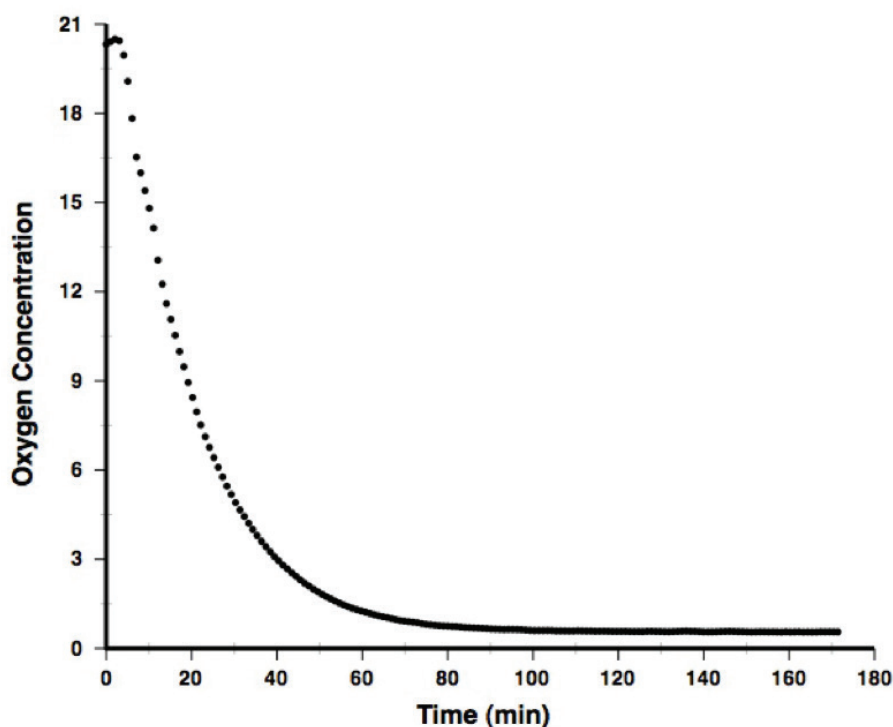
Fig.11. Oxygen concentrations in a closed respiratory chamber containing workers of *Colobopsis anderseni*. Data from Nielsen and Christian (2007).
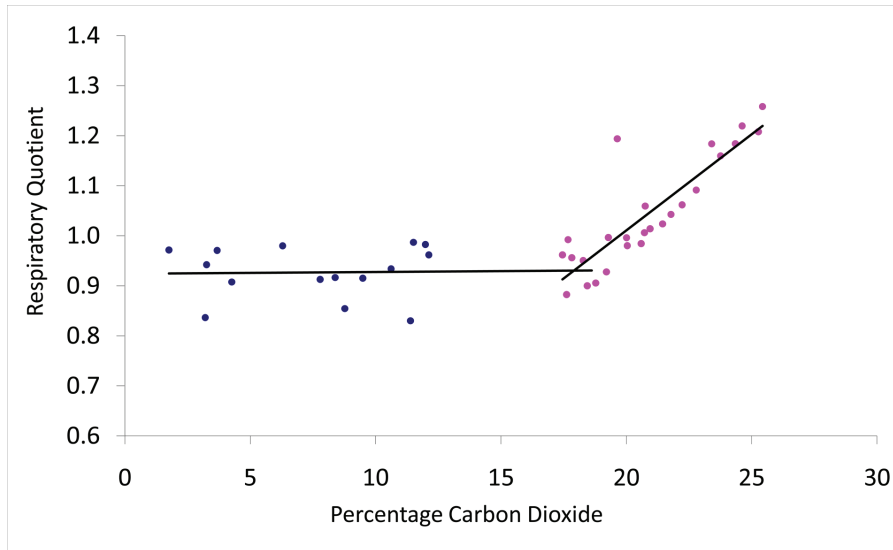
Fig.12. Respiratory Quotient (RQ) for workers of *Colobopsis anderseni* in closed respiration chambers at different CO_2 concentrations at the end of the experiment. Line (1) represents the CO_2 concentrations with constant RQ, and Line (2) shows the CO_2 concentrations associated with increasing RQ, representing increasing anaerobic respiration. Data from (Nielsen and Christian 2007)

A series of experiments were carried out to investigate the rate of decrease in the O_2 concentration in a closed respiratory chamber with *C. anderseni*. The results show that the O_2 concentration decreases linearly until it is about 18%, then there is an exponential decrease until levels reach 4% upon which the slope becomes much lower (Fig. 11) (Nielsen and Christian 2007). A similar experiment but with a CO_2 absorber in the chambers found a linear decrease until 4% O_2 which suggests the rate of decline is reduced because of the high concentration of CO_2.

Some simple and preliminary experiments of the tolerance of low O_2 and high CO_2 concentrations were conducted with *C. anderseni* and, for comparison, the arboreal *O. smaragdina*. The ants were kept in small glass tubes with moist filter paper in low oxygen or high carbon dioxide concentrations for 72 hours or until all the ants were dead. The preliminary results for the low oxygen levels were that both species could survive in 5% O_2 + 95% N_2. With 3% O_2 + 97% N_2 most *O. smaragdina* died and it is doubtful that they would survive for long at that concentration in nature. *Colobopsis anderseni* had a higher survival at 3% O_2 but more detailed investigations are necessary to determine whether they can survive normally in this concentration. In high carbon dioxide concentrations (>30%) all *O. smaragdina* died within 3 - 4 hours irrespective of the O_2 concentrations, whereas *C. anderseni* could survive 10-15 hours. With 100% N_2 the survival was the same as 3% O_2 (Nielsen unpublished data). 3 - 5% oxygen concentrations seem to be a critical concentration, when the effect of high CO_2 concentrations is absent.

The remarkable ability that *C. anderseni* shows to survive extreme atmospheric conditions suggests, that they might switch to anaerobic respiration. A series of experiments were performed to examine this hypothesis (Nielsen and Christian 2007). About 100-150 ants were placed in small double-necked Warburg chamber, which was connected to a CO_2 analyzer and with a O_2 sensor inserted into the chamber. The experiment lasted for between 15 minutes and four hours. O_2 concentrations were measured continuously, as was the amount of produced CO_2 at the end of the experiment.

The respiratoric quotient RQ is the ratio of CO_2 production and O_2 consumed. The value indicate which macronutrient are metabolized – only lipids give a value of 0.7 and protein and carbohydrate give 0.8 and 1.0, respectively. Values above 1.0 can only occur during anaerobic metabolism or during transformation of carbohydrate to lipids - which is very unlikely (Keisner and Buch

1964). The results clearly showed that anaerobic respiration occurs at levels higher than 18% CO_2 (Fig. 12). This is the first time that anaerobic respiration has been demonstrated in social insects. In a latter series of experiments *C. anderseni*, *P. sokolova* and, for comparison, the strictly aerial *O. smaragdina* were kept under anaerobic conditions and subsequently tested for lactate, the usual end product of anaerobiosis in animals. None produced lactate (Nielsen unpublished data). However, it is known that some insects produce several anaerobic end products besides lactate (Hoback and Stanley 2001). Alanine is an important end product for leaf beetles *Agelastrica alni* (Kolsch et al. 2002) and for aquatic insect larvae a significant end product are arginine, alanine, ethanol and succinate (Redecker and Zebe 1988), none of which have yet been assessed for ants.

In a further study, the changes in metabolites in these ants were investigated by using nuclear magnetic resonance spectroscopy (MRS) (Malmendal et al. 2006, Nielsen, Malmendal, and Henriksen unpublished data). *Colobopsis anderseni*, and *O. smaragdina* showed the same pattern with an increase of glucose, maltose and alanine and a decrease of coenzyme A and trehalose, whereas *P. sokolova* showed an increase in alanine and acetate. It is very interesting that the strictly arboreal ant *O. smaragdina* which never experiences inundation was able to switch to anaerobic respiration. Maybe this capability is universal within ants?

CONCLUSIONS AND REMARKS

Investigations of the insect fauna of mangroves is concentrated mainly blood-sucking Diptera, because of their transmission of diseases and discomfort for people. Their presence might also be one of the reasons for the limited number of studies of other insect groups. Ants are one of the casualties of this bias, even though they are one of the most important insect groups in the mangrove (De Baar 1993, Caitano et al. 2018). The fragmentary investigations of ants in mangroves focus on different aspects of their biology and only a few are real faunistic investigations.

There are no publications on the mangrove's ants of Africa, and there are a few from Asia, apart from papers on *O. smaragdina*. From Florida, the West Indies and South America there are several publications covering many different aspects of the biology of mangrove ants in Florida, the West Indies and South America, but none of them deal with the effects of tidal flooding. In contrast there are several faunistic and behavioral publications dealing with the biology and special adaptations to avoid drowning during inundation of Australian mangrove ants.

The distinguishing feature of mangrove ants is that they face flooding twice a day to a few times each month during the tidal cycle and have adapted to it. Other ants may be occasionally flooded after heavy rain, the flooding of rivers or after tidal surges but show no particular adaptations to these conditions (Nielsen 2011).

When most insects experience hypoxic and hypercarbic conditions, it is primarily due to conditions in the environment or microhabitat, and their exchange of respiratory gases have marginal influence on the O_2 and CO_2 concentrations (Hoback and Stanley 2001). But for nests of most ant species that are inundated, the hypoxic and hypercarbic conditions are entirely due to their own respiration.

The influence of mangrove ants on these special ecosystems is very poorly investigated, with the only exception being the studies of *O. smaragdina* in Thailand mangroves (Offenberg, 2004a, b, 2006a). These studies showed that the ants could reduce the herbivory of sesarmid crabs and Chrysomelid beetles on *Rhizophora mucronate* Lamk. leaves. Another interesting subject is the symbiotic relation between mangrove ants and other insects. Scale insects are known to have symbiosis with many twig-nesting ants in mangroves (Nielsen, 1997b, 2000), but how important are they for the food supply of the colony, and how do the scales disperse? Are there symbiotic relations with aphids or extrafloral nectaries on the mangrove trees? There are many aspects of the biology of mangrove ants that relationships with required elucidation, besides their ability to survive the harsh environmental conditions.

Also noteworthy is that most mangroves are situated in the tropics and the air temperatures can reach more than 40 ⁰C. Most of the laboratory experiments in Australia were carried out at 25 ⁰C. It was known that the temperature in the nests of *C. anderseni* can be up to 43 ⁰C. This would increase the respiratory rates about four times, thus depleting the oxygen and increasing the carbon dioxide concentration more rapidly and so prolong the period of hypoxic and hypercarbic conditions during immersion (Nielsen et al. 2006).

The very extreme conditions in the intertidal mangrove habitat are a unique place to study remarkable adaptations for the survival of insects.

ACKNOWLEDGEMENTS

REFERENCES

Alongi D, 2002. Present state and future of the world's mangrove forests. Environmental Conservation 29(3): 331 – 349.

Attenborough D, 2005. Life in the undergrowth. London, British Broadcasting Cooperation.

Bujan J, Roeder KA, Yanoviak SP and Kaspari M, 2020. Seasonal plasticity of thermal tolerance in ants. *Ecology* 101(6)

Caitano B, Dodonov P and Delabie JHC, 2018. Edge, area and anthropization effects on mangrove-dwelling ant communities. Acta Oecologica-International Journal of Ecology 91: 1 – 6.

Clay RE and AN Andersen, 1996. Ant fauna of a mangrove community in the Australian seasonal tropics, with particular reference to zonation. Australian Journal of Zoology 44(5): 521 – 533.

Cole BJ, 1980. Repertoire convergence in 2 mangrove ants, *Zacryptocerus-varians* and *Camponotus (colobopsis)* sp. Insectes Sociaux 27(3): 265 – 275.

Cole BJ, 1983a. Assembly of mangrove ant communities - colonization abilities. Journal of Animal Ecology 52(2): 349 – 355.

Cole BJ, 1983b. Assembly of mangrove ant communities - patterns of geographical-distribution. Journal of Animal Ecology 52(2): 339 – 347.

Craft C. (2015). Creating and Restoring Wetlands, Elsevier, 358pp

Crozier RH, Kaufmann B, Carew ME and Crozier YC, 1999. Mutability of microsatellites developed for the ant *Camponotus consobrinus*. Molecular Ecology 8(2): 271 – 276.

De Baar M and Hockey M, 1993. Mangrove insects. Australian Science 2: 44 – 45.

Dejean, A, Durou S, Olmsted I, Snelling RR and Orivel J, 2003, Nest site selection by ants in a flooded Mexican mangrove, with special reference to the epiphytic orchid *Myrmecophila christinae*. Journal of Tropical Ecology 19: 325 – 331.

Dejean A and Olmsted I, 1997. Ecological studies on *Aechmea bracteata* (Swartz) (Bromeliaceae). Journal of Natural History 31(9): 1313 – 1334.

Duke NC, 2006. Australia's Mangroves The authoritative guide to Australia's mangrove plants. Brisbane, University of Queensland, 200pp.

Fellowes JR ,1999. Preliminary observations of the ants in Hong Kong Mangroves. Proceedings of the International Workshop on the Mangrove Ecosystems of Deep Bay and the Mai Po Marches, Hong Kong. (ed. S. Y. Lee). Hong Kong University Press.

Giri C, Ochieng E, Tieszen LL, Zhu Z, Singh A, Loveland T, Masek J and Duke N, 2011. Status and distribution of mangrove forests of the world using earth observation satellite data. Global Ecology and Biogeography 20(1): 154 – 159.

Guofang J, 1997. A Preliminary Analysis of Species Composition and Its Seasonal Fluctuation of Insects in Shankou Mangrove Area. Journal of Guangxi Academy of Sciences 13: 11 – 17.

Hoback WW and Stanley DW, 2001. Insects in hypoxia. Journal of Insect Physiology 47(6): 533 – 542.

Hogarth PJ, 2007. The biology of Mangroves and Seagrasses. New York, Oxford University Press Inc, 273pp

Huxley C R, 1978. Ant-plants myrmecodia and hydnophytum (rubiaceae), and relationships between their morphology, ant occupants, physiology and ecology. New Phytologist 80(1): 231 – 268.

Hölldobler B and Wilson EO, 1990. The Ants. Cambridge, MA, The Belknap Press of Harvard University Press, 733pp.

Kathiresan K and Bingham BL, 2000. Biology of mangrove ecosystems. Advances in Matine Biology 40: 81 – 251.

Keister, M.and Buck, J., 1964. Respiration: some exogenous and endogenous effect on rate of respiration. In: Rockstein, M. (Ed.), The Physiology of Insecta. Academic Press, New York, London.

Kleineidam C and Roces F, 2000. Carbon dioxide concentrations and nest ventilation in nests of the leaf-cutting ant *Atta vollenweider*i. Insectes Sociaux 47(3): 241 – 248.

Kohout RJ, 1988. Nomenclatural changes and new Australian records in the ant genus *Polyrhachis* Fr. SMITH (Hymenoptera: Formicidae: Formicinae). Memoirs of the Queensland Museum 25: 429 – 438.

Kolsch G, Jakobi K, Wegener G and Braune HJ, 2002. Energy metabolism and metabolic rate of the alder leaf beetle *Agelastica alni* (L.) (Coleoptera, Chrysomelidae) under aerobic and anaerobic conditions: a microcalorimetric study. Journal of Insect Physiology 48(2): 143 – 151.

Lavelle P and Spain AV, 2001. Soil Ecology. Kluwer, Dordrecht, Nederlands. 654pp.

Lever RJAW, 1952. A Malayan leaf tying caterpillar from mangroves periodical submerged in seawater. Entomologist's Mon. Mag. 88 – 95

Lopes B L and Aguiar dos Santos R, 1996. Aspects of the ecology of ants (Hymenoptera:Formicidae) on the mangrove vegetation of Rio Ratones, Santa Catarina Island, SC, Brazil. Bol. Entomol. Venez. N.S. 11 (2): 123 – 133.

Lymburner Leo, Richard Lucas, Peter Scarth, Imam Alam, Claire Phillips, Catherine Ticehurst, Alex Held, 2020. Mapping the multi-decadal mangrove dynamics of the Australian coastline. Remote Sensing of Environment 238:111185

Malmendal A, Overgaard J, Bundy JG, Sorensen G, Nielsen NC, Loeschcke V and Holmstrup M, 2006. Metabolomic profiling of heat stress: hardening and recovery of homeostasis in Drosophila. American Journal of Physiology-Regulatory Integrative and Comparative Physiology 291(1): R205 – R212.

Murphy DH 1990. The natural history of insect herbivory on mangrove trees in and bear Singapore. Raffles Bulletin of Zoology 38: 119 – 203.

Nielsen MG, 1997a. Nesting biology of the mangrove mud-nesting ant *Polyrhachis sokolova* Forel (Hymenoptera, Formicidae) in Northern Australia. Insectes Sociaux 44(1): 15 – 21.

Nielsen MG, 1997b. Two specialised ant species, *Crematogaster* (*australis* Mayer group) sp. and *Polyrhachis sokolova*." Northern Territory Naturalist 15: 1 – 5.

Nielsen MG, 2000. Distribution of the ant (Hymenoptera: Formicidae) fauna in the canopy of the mangrove tree *Sonneratia alba* J. Smith in Northern Australia. Australian Journal of Entomology ***39***: 275 – 279.

Nielsen MG, 2011. Ants (Hymenoptera: Formicidae) of mangrove and other regularly inundated habitats: life in physiological extreme. Myrmecological News 14: 113 – 121.

Nielsen MG, Christian K and Birkmose D, 2003. Carbon dioxide concentrations in the nests of the mud-dwelling mangrove ant *Polyrhachis sokolova* Forel (Hymenoptera: Formicidae). Australian Journal of Entomology 42: 357 – 362.

Nielsen, MG, Christian K, Henriksen PG and Birkmose D, 2006. Respiration by mangrove ants *Camponotus anderseni* during nest submersion associated with tidal inundation in Northern Australia. Physiological Entomology 31(2): 120 – 126.

Nielsen MG, Christian K and Malte H. 2009. Hypoxic conditions and oxygen supply in nests of the mangrove ant, *Camponotus anderseni*, during and after inundation. Insectes Sociaux 56(1): 35 – 39.

Nielsen MG and Christian KA, 2007. The mangrove ant*, Camponotus anderseni*, switches to anaerobic respiration in response to elevated CO2 levels. Journal of Insect Physiology 53(5): 505 – 508.

Nielsen MG, Christian K, Henriksen, PG and Birkmose D, 2005. Respiration by mangrove ants *Camponotus anderseni* during nest submersion associated with tidal inundation in Northern Australia. Physiological Entomology 30: 1 – 7.

Offenberg J, Havanon S, Aksornkoae S, MacIntosh D J and Nielsen MG, 2004a. Observations on the ecology of weaver ants (*Oecophylla smaragdina* Fabricius) in a Thai mangrove ecosystem and their effect on herbivory of *Rhizophora mucronata* Lam. Biotropica 36(3): 344 – 351.

Offenberg J, Macintosh D J and Nielsen MG, 2006a. Indirect ant-protection against crab herbivory: damage-induced susceptibility to crab grazing may lead to its reduction on ant-colonized trees. Functional Ecology 20(1): 52 – 57.

Offenberg J, Nielsen M G, MacIntosh D J, Havanon S and Aksornkoae,S, 2004b. Evidence that insect herbivores are deterred by ant pheromones." Proceedings of the Royal Society of London Series B-Biological Sciences 271: S433-S435.

Olsen AR, 2009. New record of the marine littoral ant *Odontomactus maligans* Smith, F. 1859, in Palau. The pan-pacific Entomologist 85(1) 11 – 12.

Redecker B and Zebe E, 1988. Anaerobic metabolism in aquatic insect larvae - studies on *Chironomuse thummi* and *culex-pipiens*. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 158(3): 307 – 315.

Richards, D. R., Thompson, B. S., and Wijedasa, L. (2020). Quantifying net loss of global mangrove carbon stocks from 20 years of land cover change. Nature communications, 11(1), 1 – 7.

Romer D, Bollazzi M and Roces F, 2018. Carbon dioxide sensing in the social context: Leaf-cutting ants prefer elevated CO2 levels to tend their brood. Journal of Insect Physiology 108: 40 – 47.

Saenger, P, 2002. Mangrove Ecology, *Silverculture and Conservation*. Kluver Academic Publishers, Dordrecht. 360 pp.

Sousa, Camargo, Caldato, Farias, Matos, Zanuncio, Santos, and Forti 2021. Carbon dioxide levels in initial nests of the leaf-cutting ant *Atta sexdens* (Hymenoptera: Formicidae). Scientific Reports 11(1): 20562

Wang WY, Yamada A and Eguchi K, 2018. First discovery of the mangrove ant *Pheidole sexspinosa* Mayr, 1870 (Formicidae: Myrmicinae) from the Oriental region, with redescriptions of the worker, queen and male. Raffles Bulletin of Zoology 66: 652 – 663.

Wang WY, Yamada A, Yamane S. Maritime Trap-Jaw ants (Hymenoptera, Formicidae, Poneridae) on the Indo-Austalian region – Rediscription of *Odontomactus maligans* Smith and description of a related new species from Singapore, including first descriptions of males. ZOOKEYS (915), 137 – 174.

Wetterer JK 2018. Native and exotic ants (Hymenoptera: Formicidae) nesting in red mangroves (Malpighiales: *Rhizophora mangle*) of eastern Florida. Transactions of the American Entomological Society 144(2): 347 – 357.

Wetterer JK, 2018a. Native and exotic ants nesting in red mangroves of Jamaica" Transactions of the American Entomological Society 144(3): 565 – 570.

Wetterer JK, 2018b. Native and exotic ants (Hymenoptera: Formicidae) nesting in red mangroves (Malpighiales: *Rhizophora mangle*) of eastern Florida. Transactions of the American Entomological Society 144(2): 347 – 357.

Yeo, D., Srivathsan, A., Puniamoorthy, J. et al., 2921. Mangroves are an overlooked hotspot of insect diversity despite low plant diversity. BMC Biol 19, 202 (2021).

Mogens Gissel Nielsen

APPENDIX 1

List of species from mangrove investigations – species from epiphytic ramets are not included.

New World
Santa Carina, Brazil (Lopes 1996)
Azteca sp.
Linepitema sp. 4
Myrmelachista sp. 4, 5
Camponotus sp. 2, 3, 4, 9, 10
Crematogaster sp. 7, 8, 9
Hylomyrma sp.
Pheidole sp. 7, 10
Solenopsis sp. 3, 7, 8
Wasmannia sp.
Neoponera villosa
Pseudomyrmex gracillis
Pseudomyrmex sp.

Kingston Jamaica (Wetterer 2018a)
Camponotus fugax
Camponotus concpicuus
Cephalotes jamaicensis
Crematogaster vicina
Crematogaster steinheili
Tapinoma littorale
Tapinoma melanocephalum
Pseudomyrmex cubanensis
Pseudomyrmex sduardi
Pseudomyrmex tenuissimus
Pseudomyrmex simplex
Solenopsis sp.
Monomorium ebeninum
Monomorium floricola
Xenomyrmex floridanus
Trichomyrmex destructor
Technomyrmex difficilis
Tetramorium bicarinatum

Florida USA (Cole 1983a, b)
Cephalotes varians
Xenomyrmex floridanus
Crematogaster ashmeadi
Camponotus sp.
Pseudomyrmex elongatus

Florida USA (Wetterer 2018b)
Brachymyrmex patagonicus
Camponotus floridanus
Camponotus inaequalis
Camponotus planatus
Camponotus sexguttatus
Cardiocondyla obscurior
Cephalotes varians
Colobopsis impressa
Crematogaster ashmeadi
Crematogaster steinhelli
Monomorium floricola
Pseudomyrmex cubaensis
Pseudomyrmex gracillis
Pseudomyrmex simplex
Solenopsis zeteki
Tapinoma ltorale
Tapinoma melanocephalum
Technomyrmex difficilis
Wasmannia auropunctata
Xenomyrmex floridanus

Asia
Shankou South China (Goufang 1997)
Oecophylla smaragdina
Polyrhachis dives
Crematogaster dohrni artifex
Dorylus orientalis
Odontoponera transversa
Solenopsis geminata
Tetramorium bicarinatum
Crematogaster sp.
Monomorium sp.
Anoplolepis gracilipes
Camponotus dolendus
Camponotus nicobarensis
Dolichoderus sp.

Hong Kong (Fellowes 1999)
Dolichoderus sp. 3 (species near *thoracicus*)
Iridomyrmex sp.1 (*anceps* species group)
Ochetellus sp. 1
Tapinoma sp. 1
Camponotus sp. (cf. *tokioensis*)
Camponotus nicobarensis
Camponotus nipponicus
Paratrechina longicornis
Polyrhachis (*Myrmhopla*) *dives*
Crematogaster sp. 3
Monomorium chinense

Carebara diversa
Pheidole (cf. *mus*) sp. 7
Pheidole (cf. *nodus*) sp. 1
Tetramorium kraepelini
Tetraponera nitida

Ca Mau Mecon Vietnam
(Nielsen unpl., Det. Alan Andersen)
Anoplolepis gracilipes
Camponotus sp. A (*maculatus* gr.)
Cataulacus granulatus
Crematogaster sp. A
Crematogaster sp. B
Crematogaster sp. C
Dilobocondyla sp.
Dolichoderus sp. A
Oecophylla smaragdina
Nylanderia sp. A (*vaga* gr.)
Paratrechina longicornis
Tapinoma melanocephalum
Tapinoma sp. A
Tetraponera ?allaborans
Tetraponera attenuata

Ranong Thailand
(Nielsen unpl., Det. Alan Andersen)
Camponotus sp. B (*reticulatus* gr.)
Camponotus sp. C (*saundersi* gr.)
Crematogaster sp. A
Crematogaster sp. B
Crematogaster sp. D
Oecophylla smaragdina
Philidris sp. A
Polyrhachis sp. A (*tibialis* gr.)
Tapinoma sp. B
Tapinoma sp. C (*minutum* gr.)
Technomyrmex sp.
Tetraponera nitida

Darwin Harbour Australia (Clay and Andersen
1966, Nielsen 2000, Noske unpl., Hofmann unpl.)
Clay and Andersen (1996)
Crematogaster sp. 1 (*laeviceps* group)
Crematogaster sp. 2
Crematogaster sp. 3
Iridomyrmex sp. 1 (*anceps* group)
Monomorium sp. 1
Monomorium turneri
Oecophylla smaragdina
Opisthopsis major
Polyrhachis senilis
Polyrhachis constricta
Polyrhachis terpsichore
Polyrhachis sokolova
Polyrhachis sp. 5 (*euterpe* Forel group)
Polyrhachis sp. 6 (*euterpe* Forel group)
Tapinoma sp. 1
Tapinoma sp. 2

R. Noske (unpublished) and B. Hofmann (un-
published)
Tetraponera punctulata
Colobopsis anderseni
Camponotus sp. B (*janeti* group)
Monomorium floricola

B. Hofmann (unpublished)
Monomorium sp.
Opisthopsis haddoni

Nielsen (2000)
Tapinoma melanocephalum
Camponotus sp. C (*maculatus* group)
Bothroponera sp. (*porcata* group)
Crematogaster (*australis* Mayr group) sp. B

APPENDIX 2

Methods for microsatellite analyze of the genetic structure in *C. anderseni* colonies.

Branches of about 80 cm length, with a high density of ants, were chosen, cut off from their tree and all nest entrances were sealed. In the laboratory the branches were deep-frozen and a detailed map of the branch were constructed with all the internodal nests and nest entrances marked. Finally, all twigs were split and the contents of each nest were preserved in 99 % alcohol. Four branches (containing 315 nests in total) were used for the analysis of the genetic structure of the ants in the nests. DNA was extracted from about twenty workers per internode and from all alate sexuals present. When the number of adults in a nest was low, DNA was extracted from larvae, pupae and eggs. Three microsatellite loci (Ccon12, Ccon42, Ccon70) which had been developed for *Camponotus consobrinus* (Crozier 1999) were tested for variation in *C. anderseni*, using the published primer sequences for amplification (Nielsen et al. unpl.)

APPENDIX 3

Methods for measuring O_2 and CO_2.

To measure gas concentrations within *C. anderseni* nests, they were cut from the *S. alba* trees at least 8 hours after last inundation (Nielsen et al. 2006). The entrances were covered with tape to prevent the ants from leaving the nests. Holes (approximately 0.8 mm in diameter) were drilled from the ends of the twig into the nest chamber and a glass syringe with a three-way valve was used to take 2–5 ml air samples through one of the holes in the nest. The syringes were taken to the laboratory for analysis. The CO_2 concentrations were measured using a flow-through analyzer (LI-COR model LI-6251) and O_2 concentrations were measured in a small glass cell with a micro-oxygen electrode with a tip diameter of 1.1 mm. The mean $\pm$SD concentration of CO_2 in the nests was 5.5 ± 3.2 % (range 1.5 –12.5%). The very large deviations due to the variations in the content of biomass, where the mean $\pm$ SD biomass volume in the nests was 22.7 ± 9.4 % of the total nest volume (range 1.5 to 49.6 %).

Respiratory rates of *C. anderseni*, expressed as ul CO_2 per mg fresh weight per hour, was measured in whole nests in the laboratory. The entrance hole was sealed and the nest connected to a CO_2 analyzer. Six nests were used and the time for each analysis ranged from 0.5 to 240 minutes. The CO_2 concentration reached extremely high levels, over 30%. At 10 % and 25 % CO_2 the respiratory rate had decreased to 18,9 % and 1.8 % of rates in atmospheric air, respectably. A logarithmic correlation between the time the nest was sealed and the CO_2 concentration in the nests indicated that the concentrations of 10 and 20% CO_2 in the nests were reached after 22 and 140 min, respectively. All ants survived these high levels of CO_2 (Nielsen et al 2006).

In the following experiments the oxygen concentrations were measured with fiber-optic micro sensor connected to an oxygen meter (PreSens Microx TX3). The sensor could be inserted into the nest through a hypodermic needle such that the sensor tip extended 2–5 mm inside the cavity (Fig. 9)