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Ecological Traits and Genetic Variation in Amazonian Populations of the Neotropical Millipede *Poratia obliterata* (Kraus, 1960) (Diplopoda: Pyrgodesmidae) (Brazil)

Dissertation

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Diese Arbeit widme ich meiner Familie

Meinen Eltern, meiner Schwester und meinen Großeltern

und meinen besten Freundinnen

Kathleen Katscher und Swetlana Siniza

Wunderbare Menschen, die mich stets unterstützt haben
durch ihre Loyalität, ihr Verständnis und ihr Vertrauen

Whilst standing in the midst of the grandeur of a Brazilian forest,
It is not possible to give an adequate idea
Of the higher feelings of wonder, admiration, and devotion
Which fill and elevate the mind.

(Charles R. Darwin 1832)

It is not the strongest of the species that survive,
Nor the most intelligent,
But the one most responsive to change.

(Attributed to Charles R. Darwin)

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1 INTRODUCTION

1.1 Study Area

1.1.1 Geology of the Amazon Basin

Extending to the northern and southern regions surrounding the equator, the Amazon Basin covers an area of circa 7 million km^2 (Sioli 1984a), this territory corresponding to approximately two-thirds of the size of Europe. In the north, it is defined by the Guyanian shield, while in the south and west, by the Brazilian shield and the Andes, respectively. The enormous drainage basin gathers water from both hemispheres, covering most of northern Brazil and parts of Bolivia, Peru, Ecuador, Colombia and Venezuela (Fig. 1). The river Amazon, regionally termed Solimões to the point of confluence with the Negro River in Brazil (Irion et al. 1997), flows across the area in a west-east direction to meet the Atlantic Ocean. With a length of approximately 6,280 km, the Amazon is the world's second longest river, carrying more water than any other river on the globe.

The Solimões/Amazon River originates from two major headstreams, the Ucayali and the Marañón (Fig. 1), within the Andes, and is therefore rich in suspended sediments/nutrients and pH-neutral (Sioli 1984a). It is referred to as a 'whitewater' river owing to the cloudy colour of its waters. Due to the sediment load, the adjacent floodplains can be considered as a geochemical extension of the Andes and their foothill zone (Fittkau 1971). In contrast, most of the other streams carry clear water which is poor in such inorganic particles. Some of these rivers, e.g. the Negro River which has its source in a large depression to the west of the Guyanian shield, are dark and acid due to dissolved humic substances, resulting in the term 'blackwater' (Sioli 1984a). The adjacent floodplains, which encompass an area of circa 300,000 km^2 (Irion et al. 1997), i.e. a region approximately the size of Italy, are distinct in sediment and soil features according to the quality of the respective rivers. The grounds in blackwater areas are equally acid and poor in nutrients, whereas soils over whitewater terrain are in general well buffered and relatively rich in nutrients (Sioli 1984b; Furch 1997).

For most of its course, the Amazon River shows an average depth of circa 50 m. The gradient of the river is very low, with Manaus, located circa 1,610 km upstream, being only approximately 30 m higher than Belém (Fig. 1). Since a vast area (> 1 million km^2) is situated less than 100 m above the present sea-level, the geomorphology of the central and lower Amazon Basin has been shaped by sea-level fluctuations in the Pleistocene

Figure 1. Map of the Amazon Basin (Picture: www.amazondiscover.com).

(Irion et al. 1997). At its middle reaches and lower course, at present the Amazon River flows virtually within the sediments deposited between the ice ages, i.e. during high sea-levels (Sioli 1984b; Irion et al. 1997). Hence, the wide regional flood plain area is characterised by a mosaic of the main river, its tributaries and a multitude of islands, lakes and canals. That accounts for the predominantly island nature of terrestrial habitats in the whitewater floodplains (Sioli 1956), i.e. connections to non-flooded regions are extensively absent, limiting the horizontal migration facility of resident non-flying terrestrial arthropods (Adis 1992b). In contrast, due to their low sediment charge, many black- and clear-water rivers have not yet replenished the bottoms shaped in the

ice age phases. Hence, at least at their lower courses, the respective riverbeds clearly exceed the size corresponding to their current water load (Irion et al. 1997). As a result, blackwater floodplains are naturally linked to non-flooded areas by means of an elevation gradient, facilitating horizontal evasion from flood waters (Sioli 1951; Adis 1992b).

The main study area was situated in the vicinity of Manaus, Amazonia State, Brazil (Fig. 1), where the Negro River discharges into the Solimões/Amazon River (Fig. 2), thus enabling the comparative investigation of white-and blackwater floodplains. Manaus is located approximately 320 km south of the equator and circa 1,800 km inland from the mouth of the Amazon River, i.e. placed central in the Amazon Basin.

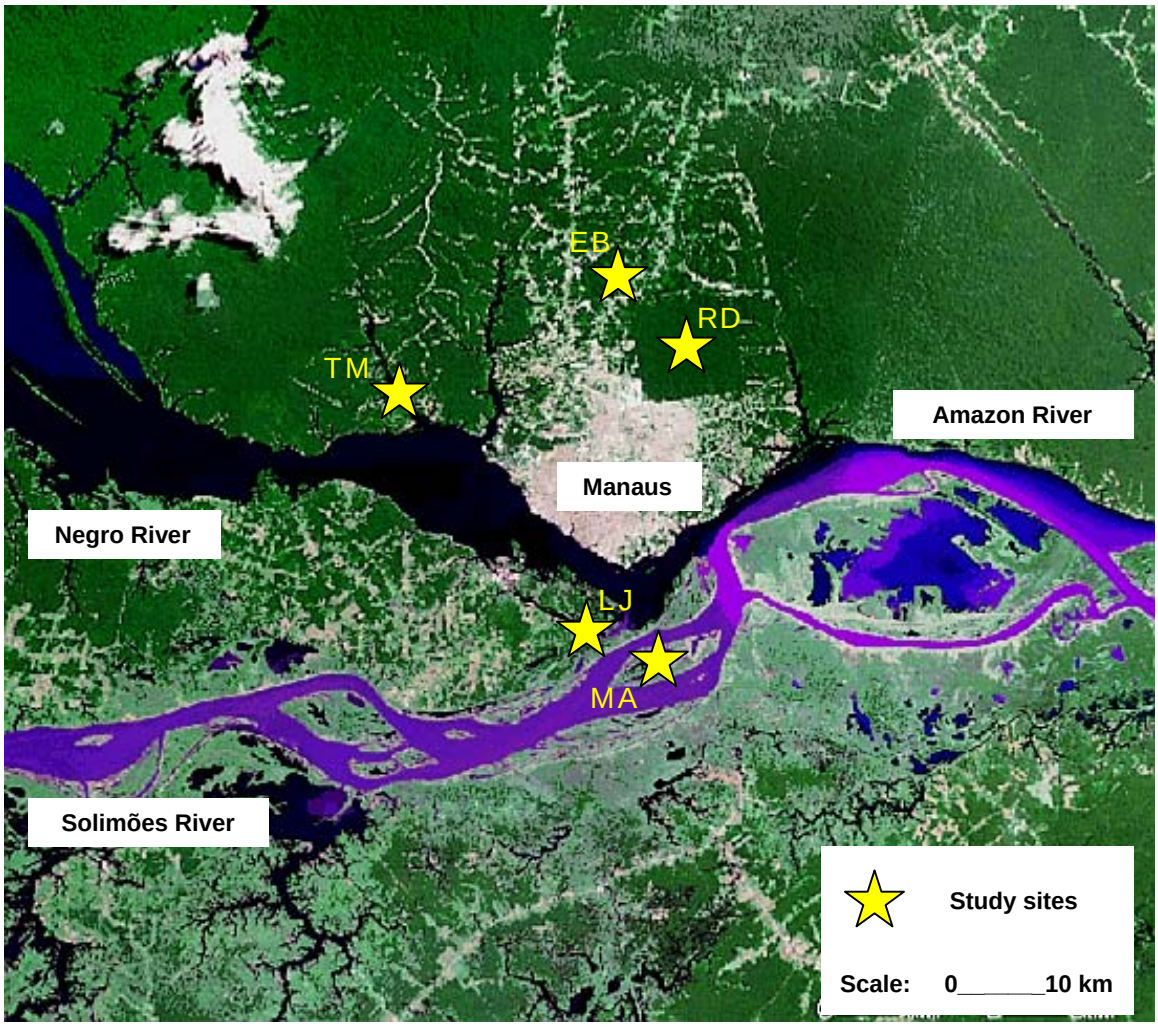

Figure 2. Study sites for collections and monitoring of *P. obliterata* in the vicinity of Manaus, AM, Brazil. Seasonal inundation forests: Marchantaria Island, MA (Várzea); Tarumã Mirím River, TM (Igapó); Lake Janauarí, LJ (Várzea & Igapó = mixedwater). Terra firme: Upland plantation at Agroflorestal Research Station CPPA/Embrapa, EB; upland forest reserve 'Reserva A. Ducke', RD (Picture: Google Earth).

1.1.2 Climate

The Central Amazonian climate corresponds the 'Ami-climate', according to Köppen (1931): the lowest monthly mean temperature is 18 °C (annual amplitude < 5 °C) and the annual precipitation ranges from 1,000 to 2,500 mm (lowest monthly precipitation: 0 to 60 mm).

The climate is hot and humid, with a mean annual temperature of 26.6 °C. The warmest months are August to November (27.2 to 27.6 °C), the coolest January to April (25.9 to 26.1 °C). As characteristic for the humid tropics, daily fluctuations in temperature exceed the seasonal ones up to > 10 °C (Irion et al. 1997).

Based on rainfall data registered at Manaus over a period of 70 years, the annual precipitation averages 2,100 mm per year (Ribeiro & Adis 1984). Ribeiro & Adis (1984) showed that Central Amazonian floodplains and non-flooded areas are subject to a rainy season that lasts from December to May (average precipitation: 1,550 mm; 258.8 ± 36.8 mm/month) and a period of low precipitation, i.e. 'dry' season from June to November (average rainfall: 550 mm; 91.8 ± 43.8 mm/month, but each month with some rain events). Precipitation is local, however, and can fluctuate considerably. The months of heaviest rainfall are January to April; the driest months are July to September (Adis 1981). During the dry season, day temperatures are higher and the differences between day and night temperatures are more pronounced (Adis 1984). Seasonally inundated and non-flooded areas can be discriminated climatically, since rainfall in the floodplain areas is distinctly lower (Ribeiro & Adis 1984).

The relative humidity is high all over the year, ranging between 75.5 % in September and 86.7 % in April. During July and August, potential evaporation can exceed precipitation (Ribeiro & Adis 1984; Irion et al. 1997).

1.1.3 Water Level Regime

In Central Amazonia, there are seasonal changes in water level of the rivers Solimões, Amazon and Negro, which average approximately 10 m (Amaral et al. 1997). They are caused by the alternation of rainy and dry seasons in the drainage area as well as the snowmelt in the Andes (Irion et al. 1997). At Manaus, Brazil, these annual fluctuations can result in differences up to 14 m between high and low waters of the Negro River (Adis 1984). Therefore, the adjacent forest areas along the three rivers, representing approximately 2 % of the Brazilian Amazon region (Prance 1979), are inundated to a

depth of several meters for 5 to 7 months each year (Amaral et al. 1997), i.e. from March/April to August/September, depending on terrain elevation and the height of the annual flood (Adis 1981; Junk et al. 1989). Since the duration of submersion varies according to altitude, lower parts are inundated for more than six months, whereas some higher parts always remain non-flooded (never flooded upland areas). These natural events, referred to as 'monomodal flood pulse' due to the pronounced seasonal periodicity (Junk et al. 1989), have occurred in the area for at least 2.4 million years (Irion et al. 1997).

Due to regionally different rainy and dry seasons in the drainage basin, the rainy season and floodwater in the environs of Manaus are phase-delayed for approximately 4 to 6 weeks, with precipitation preceding water level rise (local rainfall has virtually no impact on the water level) (Irion et al. 1997).

1.1.4 Seasonal Inundation Forests

There are eight main types of vegetation on the inundated grounds in Central Amazonia, six of which are periodically flooded by annual cycles of rivers and two which are permanent swamp forests. They have been defined both by the vegetation cover and by the type of water or the duration of flooding (Prance 1979; Amaral et al. 1997), because the species composition seems to depend upon soil quality (i.e. available nutrients) and length of submersion (Adis 1984).

In the region of Manaus, Brazil, where the blackwater river Negro and the whitewater river Solimões meet to form the Amazon River (Fig. 2), the bordering seasonal inundation forests are influenced by the respective types of water. According to Prance (1979), the forests adjacent to the Solimões/Amazon River are called 'seasonal Várzea' or whitewater inundation forests (e.g. on Marchantaria Island, Fig. 2), while those along the Negro River are termed 'seasonal Igapó' or blackwater inundation forests (e.g. at Tarumã Mirím River, Fig. 2). The 'seasonal Várzea & Igapó' or mixedwater inundation forest is flooded by both water types (e.g. at Lake Janauarí, Fig. 2). Non-flooded upland areas are locally named 'Terra firme'.

Near Manaus, relatively high seasonal inundation forests, reaching some 30 m in height, are stocked on more elevated floodplains (> 22 m above sea-level), where the aquatic phase averages 5 to 7 months per year, i.e. from March/April to August/September (Adis et al. 1996a; Adis 1997; Worbes 1997). In contrast, white- and mixedwater

inundation forests in the vicinity of Nauta, Peru, are annually flooded for approximately three months only, i.e. from March to May (Kalliola et al. 1993). In years with a low maximum flood, the higher parts of the forests in the Manaus region (e.g. Lake Janauarí, > 26 m above sea-level, Fig. 2) are only flooded for short periods (< 90 days) or not at all (Vohland & Adis 1999).

1.2 The Millipede *Poratia obliterata*

1.2.1 Classification and Biology

1.2.1.1 Systematics

Millipedes (Myriapoda, Diplopoda) seem to contain as many as 80,000 species, of which only 11 to 12 % have been described so far (Hoffman et al. 2002). They represent one of the largest classes in the Animal Kingdom, more precisely the third largest within the Arthropoda following the Insecta and the Arachnida (Golovatch et al. 1995). However, when compared to the insects, which encompass several million species, the Diplopoda forms a relatively small taxon (Westheide & Rieger 1996). Due to meso- to hygrophily, most of millipede taxonomic richness and diversity as well as life-forms (i.e. ecomorphotypes) are encountered in the tropical and subtropical regions (Golovatch et al. 1995).

Polydesmida is the largest order of millipedes and includes more than 2,700 species (Hopkin & Read 1992). With currently 173 nominal genera assigned, the predominantly tropical Pyrgodesmidae represents one of the largest families within the Polydesmida and Diplopoda (Golovatch & Sierwald 2001). Pyrgodesmids are small animals, ranging in size between 6 and 10 mm, and are often cryptic in both mode of life and colouration. Their taxonomy is considered highly problematic (Hoffman 1979; Hoffman et al. 2002). Many of the mostly monotypic genera have been based on trivial structural differences in peripheral characters, with numerous type species being known from juveniles or females only. This can be explained in part by the relative frequency of parthenogenesis, more precisely thelytoky, i.e. the siring of female offspring from unfertilised females in this group (Enghoff 1978; Westheide & Rieger 1996). Hence, relatively few species are characterised by adequate descriptions of species-specific male genitalia, i.e. gonopods (Chapter 1.2.1.2), often the only reliable means for a precise taxonomic identification in millipedes (Hopkin & Read 1992). In addition, some species appear to be distributed by man throughout the tropics (Chapter 1.2.1.6), thus

described under different names several times from different localities. The small size and complexity of gonopod structure further complicate the study of pyrgodesmids (Hoffman 1979; Hoffman et al. 2002). As a result, the status of the genus *Poratia* Cook & Cook, 1894 had remained unsettled (Hoffman 1979) until redefined by Golovatch & Sierwald (2001). Based on the thelytokous type species *P. digitata* (Porat 1889), which was formerly referred to as *Scytonotus digitatus* Porat, 1889 (cf. Cook & Cook 1894), the identity of the genus could only be clarified by the recent discovery of topotypic (= European hothouse) male material (Jeekel 1970; Hoffman 1979; Adis et al. 2001; Golovatch & Sierwald 2001). *Poratia* is now seen as originally Neotropical, i.e. Central and northern South American, and at present it comprises seven species which were earlier assigned to various subjective synonyms (Golovatch & Sierwald 2001). One of them is the mainly Amazonian (Chapter 1.2.2) *P. obliterata* (Kraus, 1960) which was previously referred to as *Muyudesmus obliteratus* Kraus, 1960 (Kraus 1960; Shelley et al. 2000).

1.2.1.2 Basic Anatomy

The millipede body consists of three main parts, namely the head, the trunk and the telson. The head is roundish and bears a pair of antennae, two mandibles, a so-called 'gnathochilarium' (modified maxillae), eyes/ocelli (if present) and a number of sensory structures (Fig. 3a). For instance, chemo-receptors are located at the end, while the probably olfactory Tömösvary caudolaterally, of the antennae. The head capsule is usually heavily calcified (Schubart 1934; Attems 1937; Hopkin & Read 1992).

The body is generally long and cylindrical, often band-shaped in dorsal view due to lateral tergital protuberances (paranota, or paraterga), e.g. in pyrgodesmids (Attems 1940). It is mostly heavily armoured by a calcified exoskeleton. The torso, except for the first four segments (including the collum or head plate), consists of characteristic double segments, or 'diplosegments', from which the term 'Diplopoda' derived (Fig. 3a). Each double segment bears two leg pairs (Attems 1937; Hopkin & Read 1992). Although referred to as 'millipedes', the maximum number of limbs per specimen is restricted to approximately 350 leg pairs (Marek & Bond 2006). The diplosegments are composed of pro- and metazonites (Westheide & Rieger 1996). In polydesmidan millipedes, the tergites, pleurites and sternites are fused into a complete ring structure (Attems 1937). The prozonites are often conically narrowed to fit into the anterior

metazonites (like a telescope), while the dorsally lying tergites are always longer than the abdominal sternites, thus enabling the rolling of the body into a spiral or sphere (Hopkin & Read 1992).

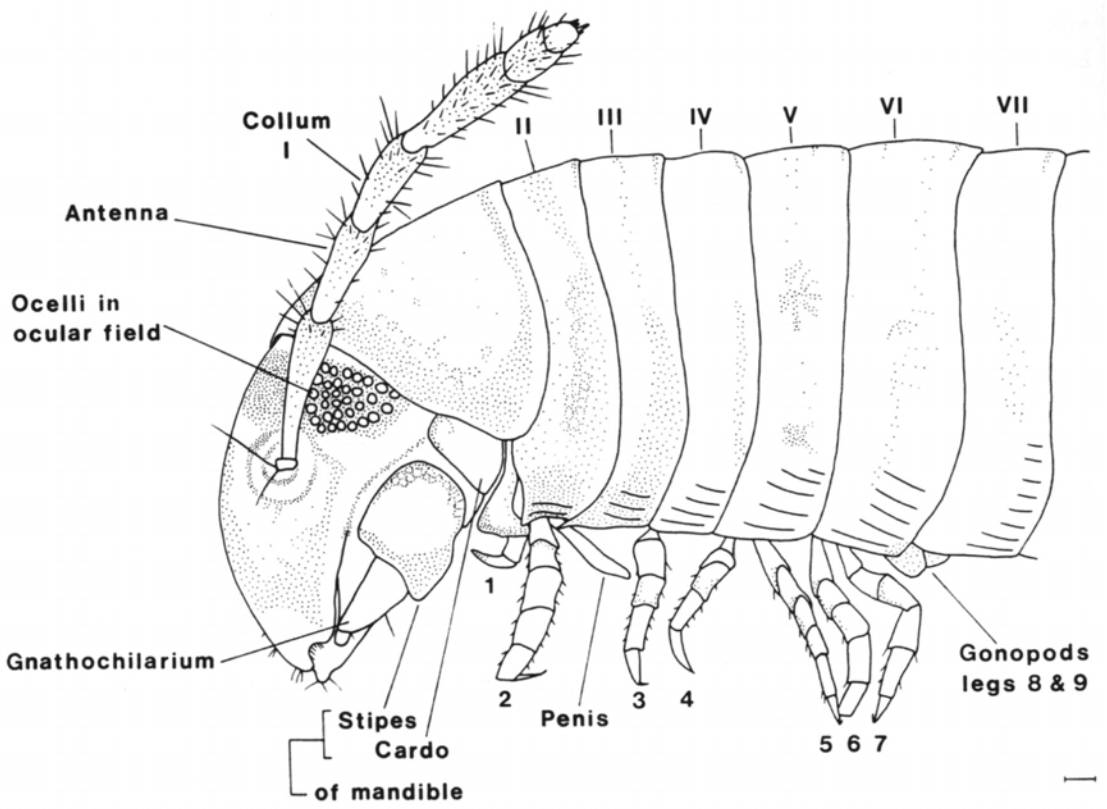

Figure 3a. External morphology of millipedes: Head and anterior body segments (I-VII) of a male *Cylindroiulus waldeni* from Madeira. Scale bar = 0.1 mm. Original drawing by H. Read (Source: Hopkin & Read 1992).

The telson consists of a pre-anal ring (often with a dorsal projection, or epiproct), a pair of anal plates that form the anal valves (paraprocts), and a subanal scale (hypoproct) (Fig. 3b). Between the telson and the posteriormost leg-bearing segment there are one or more apodous rings, followed by the proliferation zone, where new segments are initiated and develop (Attems 1937; Hopkin & Read 1992).

The animals are dioecious, with the genital opening situated in the third segment. The males lack one or both pairs of legs on the seventh segment, which are modified into fragile ‘gonopods’ (copulatory legs) often concealed inside protective pockets in the lumen of this body segment. These structures form the apparatus by which sperm is introduced into the female, thus representing spermatopositors (Attems 1937; Hopkin & Read 1992).

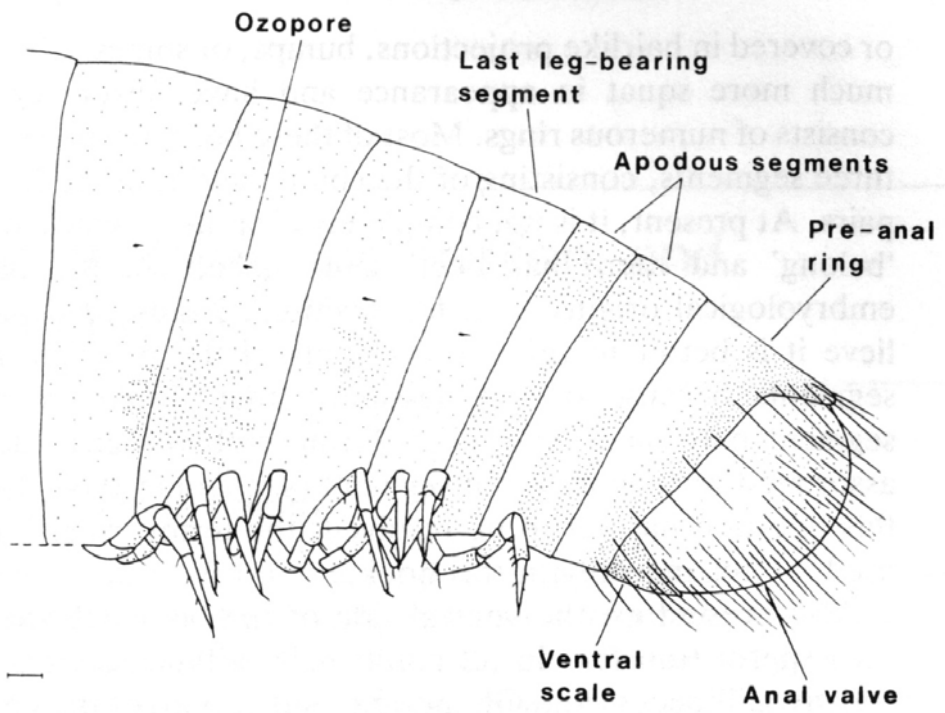

Figure 3b. External morphology of millipedes: Posterior end of a male *Cylindroiulus waldeni* from Madeira. The pre-anal ring is also known as the telson. Scale bar = 0.1 mm. Original drawing by H. Read (Source: Hopkin & Read 1992).

1.2.1.3 Characterisation

P. obliterata is a small, rather pallid to brown-pinkish pyrgodesmid (band shaped, see above) with a body length of 5 to 7 mm and 20 body segments in adults (Fig. 4). Parthenogenetic females tend to be larger than sexual ones. The width of adults ranges from 0.63 to 0.70 mm in males and from 0.67 to 0.77 mm in females. The head is roughly granulated all over the vertex and eyes are absent. The collum (head plate) is flabellate, covering most of the head from above but not in lateral view; it shows ten deeply incised, somewhat upright lobulations at the front edge. The gonopods are relatively simple regardless of population (Kraus 1960; Adis et al. 2001; Golovatch & Sierwald 2001). A detailed taxonomic diagnosis of the species can be found in Golovatch & Sierwald (2001).

1.2.1.4 Development

Only few millipedes perform brood care (Hoffman 1982). Their eggs are often deposited inside egg chambers for physical protection, mainly constructed by the female using its own excrements and saliva (Kaestner 1993; Voigtländer 2000). This is also

observed in *P. obliterata* (Fig. 5a-b). Juveniles hatch with few, mostly seven, segments and generally three leg pairs in the first developmental stage (Attems 1937). In *P. obliterata*, such hatchlings emerge 8 to13 days following oviposition (Fig. 5c) and leave the egg chamber by perforating its lateral wall (Adis et al. 2001).

Figure 4. The Neotropical millipede *Poratia obliterata* (Kraus, 1960) (Diplopoda, Pyrgodesmidae): adult female (size: 7 mm) building the basement of an egg chamber (Picture: N.G.R. Bergholz).

Millipede hatchlings acquire more segments as well as legs during their postembryonic growth, a process known as anamorphosis. The original mode of development in Diplopoda is hemianamorphosis, i.e. ecdysis (moult) without segment multiplication follows a certain number of stages with segment increase. However, *P. obliterata* develops according to a secondarily derived mode, named teleanamorphosis, where both segment number increase and ecdysis cease with maturation (Enghoff et al. 1993). *P. obliterata* comprises eight developmental stages, the final, adult one with 20 segments. Cultured specimens of populations originating from Tabatinga and Manaus, Brazil as well as from Nauta, Peru, needed 74, 68, and 63 days on average, respectively, to reach maturity (Adis et al. 2001). According to Adis et al. (2001), this postembryonic development of 2 to 3 months of duration is the shortest yet recorded among millipedes, but then, the biology of small tropical species is still poorly known. In general, millipedes take one or two years to mature, whereas the length of development can be correlated with the type of biotope/habitat (Hopkin & Read 1992).

Figures 5a-c.: *Poratia obliterata*. **a** – female (7 mm body length) with almost completed egg chamber (approx. 2 mm in diameter) with funnel-shaped ventilation tube (chimney) on top; **b** – eggs (approx. 0.3 mm in diameter) deposited within the chamber; **c** – newly hatched immatures (first developmental stage; approx. 1 mm body length) within the egg chamber (Pictures: N.G.R. Bergholz).

Millipedes are long-lived animals in comparison to most other terrestrial arthropods (Hopkin & Read 1992). However, for *P. obliterata*, the life-history is shorter than a year (Hoffman et al. 2002). Longevity of reared non-parthenogenetic females of *P. obliterata* from Manaus, Brazil and Nauta, Peru was up to 10 months only when kept without males, i.e. without laying eggs (Adis et al. 2001).

1.2.1.5 Ecology

Millipedes occur on all continents except Antarctica (Hoffman et al. 2002). Some species, due to their advantageous mode of life/life-form, show vast distributions, but most others are locally distributed (Kime & Golovatch 2000). The preferred biotopes of millipedes are forests, where they dwell under fallen leaves and bark or stones on the surface of the soil, or below the surface among soil particles. There are also several tree climbing species. In habitat choice, humidity and temperate seem to be decisive factors, whereas food and soil features appear to be less important (Kaestner 1993; Kime & Golovatch 2000).

The ‘doubling up’ of leg pairs, resulting in a larger number of legs for a relatively shorter body, enables millipedes to exert a considerable forward thrust. This, together with the calcified head capsule and leverage provided by the labrum, allows them to force their way between fibers of rotting wood and closely packed soil particles. By that manner, they can enter microhabitats that may be unavailable to other terrestrial invertebrates (Hopkin & Read 1992). There are five main ecomorphological types of millipedes, namely bulldozers or rammers, borers, rollers, bark dwellers and wedge types. The last group is typified by the polydesmids such as *P. obliterata*. These millipedes have relatively short bodies and short legs. Each postcollar segment is expanded laterally into paranota, or keels, these being directed ventrolaterally as characteristic of Pyrgodesmidae. The anterior body end is tapered so that the animal can put its head into a crevice. The legs then push upwards by straightening, causing the crevice to widen. This allows further penetration of the anterior body end. This type of burrowing is useful for a life among decaying leaves (Hopkin & Read 1992).

The cuticle of most millipede species is permeable to water and restricts their biotopes to areas where humidity is high (Edney 1977). The opposite effect, i.e. the absorption of too much water through the cuticle, may be a problem in environments subjected to flooding (Hopkin & Read 1992). Indeed, *P. obliterata* specimens from populations living in seasonally flooded forests in Brazil are not capable of enduring permanent flooding and have to retreat to the non-inundated trunk region (Adis 1986). However, they are more tolerant to inundation than individuals originating populations from non-flooded forests, since their maximum survival time underwater represented 68 hours, as opposed to only 24 hours for specimens from upland population (Wilck 2000). Very few wetland millipede species are able to survive submersion for several months, with resistance mostly being restricted to juvenile and subadult stages (Adis 1986; Adis & Messner 1997; Adis et al. 1998).

While their mostly cryptic mode of life and their largely solid, calcified tegument appear to provide an efficient protection against enemies, millipedes tend to roll into a spiral or sphere in situations of stress or danger, thereby hiding their legs inside. At the same time, when irritated, they secret toxic or repelling liquids (carboxyl acids, benzyl acids or hydrocyanic acids) from their paired defensive glands. Millipede poison can be dangerous, especially the cyanide-containing one of polydesmids (Schubart 1955; Hoffman et al. 2002). Nevertheless, millipedes have a lot of enemies, such as ants, toads

and birds of prey (Schubart 1955). In addition, they often suffer from parasites, e.g. nematodes and mites (Kaestner 1993).

Being slow, heliophobous (light-avoiding) and moisture requiring detrivores, the vast majority of millipedes nourish from decaying substances of plant and animal origin (Hopkin & Read 1992). Only few millipedes feed on living plants, usually on the soft and easily digestible parts (e.g. Bailey & Mendonca 1990). Other species nourish exclusively on the algae growing on the bark of trees (Mahsberg 1996). The particular diet of a millipede can alter during development, with juveniles feeding on specific plant roots as well as fungi and adults living on detritus (Adis 1992a; Adis & Victoria 2001). Some millipedes show a clear preference for leaf litter derived from particular tree species (Kheirallah 1978). Furthermore, Kheirallah (1979) found that each leaf had to attain a certain age before it became palatable to the animals. These preferences are possibly related to the level of defensive chemicals and the concentration of nitrogen, carbohydrates, calcium and moisture in leaves. Also important is the state of decay, since bacteria and fungi may increase the availability of nutrients (Hopkin & Read 1992).

Since almost all millipedes are primary decomposers of leaf litter, decaying wood and duff, they bear an ecological importance, particularly in the tropics and subtropics (Golovatch et al. 1995; Westheide & Rieger 1996). The main role of detritivorous invertebrates in enhancing the decomposition of dead plant remains is to stimulate microbial activity (Price 1988). The fragments of a leaf that are voided in their faeces have a greater surface area available for colonisation of bacteria and fungi than the original leaf material (Sakwa 1974; Hopkin & Read 1992). There are a few biotopes in which millipedes are responsible for ingesting more than 5 to 10 % of the annual leaf litter fall., where earthworms or termites, which are usually the dominant detritivores, are scarce, millipedes may occur at densities of several hundreds per square meter and consume 25 % of the annual litter fall (Blower 1970b; Van der Drift 1975; Bocock 1983; Mahsberg 1997). Furthermore, the need of millipedes in calcium accumulation (the only other soil arthropods to have a calcified exoskeleton are the isopods) means that they are an important component in the cycling of this essential element in terrestrial ecosystems (Seastedt & Tate 1981; Cromack et al. 1977). A preference for dead wood, the richest source of calcium, was observed in an African species which represented the dominant fragmenter at suitable sites and thus may process nutrient input into the local forest soil (Mahsberg 1997). The same could apply to some

polydesmidan millipedes resident in Central Amazonian forests, which were shown to strongly accumulate calcium from ingested wood (Vohland et al. 2003).

1.2.1.6 Dispersal Ability

Due to their limited natural dispersal capacities, a large number of endemic species has evolved in many areas of very restricted ranges. On the one hand, several of these species are particularly vulnerable to small scale environmental changes and hence may be threatened by human activities. On the other hand, the reduced vagility of millipedes has also led to their accidental dispersion in timber, plant material and soil by man, fundamentally altering the distribution pattern of many species. The frequency with which tropical species turn up and thrive in heated glasshouses in temperate countries is testimony to this fact (Hopkin & Read 1992). *P. obliterata* also represents a typical example (Chapter 1.2.2) for such a kind of introduction (Golovatch et al. 2001). Several carried-over species are also agricultural pests, damaging various crops on fields and plantations (Hoffman et al. 2002).

1.2.2 Origin and Range of *P. obliterata* Populations

The study animal *Poratia obliterata* (Kraus, 1960), a small Neotropical millipede probably originating from the Andes, is both widespread and abundant in the Amazonian region of Peru, Brazil and Colombia and it also occurs in Central America, i.e. Costa Rica and Panama (Golovatch & Sierwald 2001). This trans-Amazonian millipede was first recorded from Muyu Island in the Amazon River near Iquitos, Peru (Fig. 1), where it appeared to be the most common and frequent species compared to the co-occurring *P. insularis* (Kraus 1960). In the environs of Manaus, Brazil (Fig. 2), *P. obliterata* is likewise much more common than the latter sympatric congener (Bergholz et al. 2005).

The Solimões/Amazon River is known as a classical, unidirectional dispersal agent for the terrestrial arthropods inhabiting the adjacent, periodically flooded banks (Junk et al. 1989). Given that resident millipedes mainly survive flooding by climbing tree trunks to escape the rising waters, they may be transported downstream on floating vegetation, in particular tree trunks (Adis 1997; Vohland & Adis 1999). Such a passive pathway of dispersal could account for the occurrence of *P. obliterata* from Nauta (at the Marañon River to the west of Iquitos), Peru, down to Belém, Brazil at the mouth of the Amazon

River (Fig. 1), from where it could have been transported to Central America as well as Europe by trade channels.

Moreover, the widespread millipede is known from a remarkably wide range of biotopes, such as white-, mixed- and blackwater inundation forests, primary and secondary upland forests (in these upland forests, the species is apparently rare and never abundant, Golovatch, pers. commun.) and plantations in the environs of Manaus, Brazil (Adis et al. 2001). In other words, it inhabits biotopes with quite different ecological conditions. Due to its evident capacity to cope with seasonally flooded environments, *P. obliterata* is able to colonise both non-flooded and temporarily inundated areas, sometimes even in high abundances. The frequently occurring changes of meandering rivers in Peruvian Amazonia (Salo et al. 1986) may have forced former upland populations of *P. obliterata* to endure the transformation of the biotope into floodplain forests and vice versa within a relatively short time. The same has been postulated for other resident polydesmidan millipedes, namely *Pycnotropis tida* (Aphelidesmidae) (Bachmann et al. 1998; Vohland & Adis 1999). During a subsequent downstream colonisation of the Solimões/Amazon River, ecological adaptation and diversification processes in the respective populations may have proceeded.

Resistant thelytokous populations of *P. obliterata* withstanding the rather harsh conditions in some European hothouses are also evidence for the species' ability to get adapted to new environments. *P. obliterata* is only represented by bisexual populations throughout Amazonia, i.e. from Nauta, Peru, down to Belém, Brazil, as well as in Costa Rica and possibly Panama (Shelley & Golovatch 2001). Yet, the species comprises a parthenogenetic form since its introduction to Europe, i.e. in hothouses at the botanical gardens of Kiel, Germany (Adis et al. 2001) and Paris, France (Adis & Golovatch unpubl.), where it appears to coexist with the likewise thelytokous *P. digitata* (Golovatch & Sierwald 2001). Hence, *P. obliterata* displays a classical example of geographical parthenogenesis (cf. Enghoff 1994), with bisexual populations occurring in the area of origin and a thelytokous form in extreme, peripheral regions, i.e. European hothouses and probably open habitats in the southern USA (Adis et al. 2001; Golovatch & Sierwald 2001; Shelley 2004). Historically, these introductions are likely to have occurred with shipped plant material taken. The subsequent gradual cooling en route might have induced adaptive thelytoky in some surviving females, probably through activation of some special bacterial agents other than *Wolbachia* (Adis et al. 2001; Witzel et al. 2003). Parthenogenesis features some reproductive advantages. Fewer

founder females are required for colonisation since all individuals are capable of producing numerous offspring, i.e. direct transmission of a successful genotype to progeny without any costs of mating is possible (Enghoff 1978; Norton et al. 1993). Disadvantages of genetically identical clones, however, are the susceptibility to parasites and diseases as well as, at least in *P. obliterata*, sharply prolonged ontogeny (Adis et al. 2001).

1.2.3 Biotope-Related Adaptation and Speciation?

According to Schwerdtfeger (1963), 'habitat' refers to the specific place or environment where a plant or animal naturally lives and grows, e.g. the litter in a forest, while 'biotope' is related to a region uniform in environmental conditions and in its populations of animals and plants (= 'biocenosis') for which it is the area of settlement, e.g. a tropical upland forest.

Traits that enable the survival in a given biotope, i.e. enhance the local fitness of an organism, are referred to as 'adaptations' (Schäfer 1992; Reeve & Sherman 1993; Rose 2001). They result from selection that either occurred under similar environmental conditions to meet the current purpose or took place on other terms and/or for different purposes (Reeve & Sherman 1993; Rose 2001). The latter is often named 'preadaptation' (Schäfer 1992).

Specific adaptations, i.e. survival strategies (Chapter 1.3), in terrestrial invertebrates are required for their occurrence in periodically inundated biotopes. Ecological investigations in the study area revealed that several arthropods of upland forests, such as the polydesmidan millipede *Pycnotropis tida*, must have undertaken secondary immigrations to inundation forests (Adis et al. 1988; Adis 1997; Adis et al. 1997; Adis & Messner 1997; Bachmann et al. 1998). A prerequisite for this colonisation, they have adapted to the regularly flooded biotopes, for instance by means of univoltine life cycles (Chapter 1.3), a process in several cases accompanied by speciation. The same might well apply to *P. obliterata*.

According to the 'biological species concept', the event when one population of interbreeding organisms splits into two reproductively isolated groups of organisms is called 'speciation' (Mayr 1963; Sokal & Crovello 1970; Ridley 1996). It is still a matter of controversy whether new species evolve only from subpopulations that are geographically separated (allopatric), or also from subpopulations that are contiguous

with (parapatric), or overlap (sympatric), with the ancestral population (Mayr 1963; Endler 1977; Irwin et al. 2001; Mallet 2001; Doebeli & Dieckmann 2003). Sympatric and, usually, parapatric speciation both require reinforcement, i.e. an enhanced reproductive isolation by natural selection: forms are selected for mating with their own, not another, type if hybrids are disadvantageous (Ridley 1996). As initially proposed by Darwin (1859), the ecological species concept implies that selection due to ecological patterns provides the basis for such a process (Ridley 1989; Schluter 2001). Here, a species occupies an 'adaptive zone' (Simpson 1953), and natural selection favours interbreeding with organisms adapted to at least approximately the same niche (Ridley 1996). Recent investigations of several taxonomic groups undergoing diversification suggest that adaptation in the context of a biotope/habitat template may play a central role in this process (Schluter 1996). Ecological speciation occurs, when divergent selection for traits (morphological, physiological or behavioural) between populations or subpopulations in contrasting environments (referring to biotic and abiotic elements of the biotope/habitat) leads directly or indirectly to the evolution of reproductive isolation (Schluter 2001). Reduced hybrid fitness due to, for example, the intermediate phenotype being susceptible to predation or parasitism, or less efficient in search for food, reinforces ecological diversification processes (Schluter 2001). Therefore, ecological adaptations are deemed capable of producing discrete species. Accordingly, the periodical flood pulse in Central Amazonian inundation forests is assumed to effect ecological differentiation in some of the resident species (cf. Adis 1997). For instance, the evolutionary centre of Carabidae is believed to be located in the floodplains along tropical rivers (Erwin & Adis 1982). Here, the acquisition of annual periodicity in response to a seasonal inundation period of six months can be viewed as a basic requirement for the later colonisation of temperate zones.

In general, millipedes have long been observed as being inclined for speciation and local endemism (Verhoeff 1928-1932; Loomis & Schmitt 1971; Golovatch 1997b). Some of the reasons for this are low vagility, short life cycles and the dependence of most species on a humid microclimate, all of which easily result in isolation (Hopkin & Read 1992). Small millipedes cover only minor distances during their life spans and are therefore fairly local. Thus, it is due to their limited dispersal capacities that they exhibit a great potential for speciation. Particularly in the tropics, where their highest biodiversity is to be found, they tend to develop new life-form types and species (Hopkin & Read 1992; Enghoff 1994; Golovatch et al. 1995). This is in accordance

with the idea that stronger genetic differentiation is generally reported for species with reduced dispersal capacities, as revealed by a meta-analysis of different taxa (Bohonak 1999).

With regard to the eurytopic, or euryoecic, distribution of *P. obliterata* in different terrestrial biotopes, one can postulate the existence of different ecological or 'biotope-specific races' (Wolf & Adis 1992), maybe even subspecies. Specimens of the hypothesised ecotypes are morphologically indistinguishable (Adis et al. 2001; Golovatch & Sierwald 2001), but are likely to differ in their bionomical aspects (Chapter 1.3).

Crossing experiments between cultured males and females (both ways) originating from whitewater inundation forests at Tabatinga, Brazil and Nauta, Peru (March 1998, leg J. Adis and S.I. Golovatch) and from an upland palm tree plantation near Manaus, Brazil (October 1998, leg J. Adis) resulted in viable offspring (Adis et al. 2001). Still, ecological selection in the field might impair such outcrossing between populations from flooded and non-flooded biotopes, and thus may have induced a process of ecological speciation.

However, different life strategies can be either the result of genetic adaptations to different environments or the response of populations through phenotypic plasticity to different conditions (West-Eberhard 1989). The occurrence in these ecologically diverse biotopes can thus also reflect the ecological plasticity of a remarkable euryoecious species.

There have to date been no special studies concerning the life history and behaviour of different *P. obliterata* populations in Central Amazonia, nor have there been any analyses of probable ecological differentiation between and/or genetic variability within such populations. By testing whether the colonisation of different seasonal inundation forests resulted in adaptation or even ecological speciation in the respective populations, or if mere ecological plasticity enables the species' occurrence in various different biotope types, a better understanding of the adaptation and speciation processes in Central Amazonia can be obtained. Due to vast distribution and occurrence in a wide range of biotopes, the eurytopic millipede *P. obliterata* is particularly qualified as such a model organism. This is especially true because this millipede is one of 11 local millipede species (total millipede fauna ≥ 110) occurring in both floodplain and non-inundated areas (Adis & Junk 2002; Hoffman et al. 2002). Thus, it is of primary interest

to clarify the ecological adaptations and the genetic/systematic rank of the postulated ecotypes in *P. obliterata*.

1.3 Survival Strategies in Central Amazonian Floodplains

The climatic and biotic conditions at any geographic location change through time, because of climatic trends driven by fluctuations in the earth's orbit (Hays et al. 1976). Environmental variation is often an important aspect in the ecology of organisms, with most populations experiencing at least seasonal changes (Fretwell 1972).

Whereas elsewhere in the tropics seasonality is mostly confined to rainy and dry seasons, the Central Amazonian region is mainly influenced by the annual water level alterations of the Amazon River and its tributaries (Chapter 1.1.3). Due to this 'monomodal' flood pulse, which accounts for water level alterations of on average 9.8 m, vast forest areas along the river banks are seasonally inundated for several months a year. Hence, seasonal Amazonian inundation forests exhibit an aquatic phase with the forests floor being submerged and a terrestrial phase when the water level is low (Adis 1992b, 1997). This local change from terrestrial to aquatic phases is considered to have occurred for a period of at least two million years (Irion et al. 1997). In the affected areas, i.e. the zone between the exclusively aquatic and terrestrial biotopes, the periodic flood pulse is the main factor controlling the ecosystem (Junk et al. 1989; Adis 1992b). As a result of disturbance, the species diversity in terrestrial invertebrates is poorer in the riverine lowland compared to local upland areas (Adis & Junk 2002). The pronounced periodicity and, consequently, predictability of this natural phenomenon, however, enabled long-term acclimatisation of the resident fauna and flora (Junk et al. 1989).

Stressful environments bring about distinct adaptive patterns, at both the molecular and organismal levels, more sharply and more clearly than in non-stressful ecologies (Nevo 1990). To avoid extinction, a species living in a moving/changing habitat must either track its old habitat spatially or get locally adapted to the new environmental conditions. These dual processes of migration and evolution also determine the biogeographic pattern of species (Brown & Gibson 1983). The presence or absence of local adaptation can often be related to the species' vagility (Gandon et al. 1996). Migration is maladaptive when the environment is variable in space (Balkau & Feldman 1973; Holt

1985), but, when the environment is variable in time, this is not necessarily the case (Gandon et al. 1996).

Adaptations of organisms to diverse adverse ambiental conditions (i.e. stress factors) that increase their survive capacities are referred to as survival strategies, including, amongst others, avoidance, dormancy and migration (Tischler 1984; Schäfer 1992). The strategies are genetically determined behavioural patterns and thus do not imply a conscious action by the respective organisms (Southwood 1977; Chapleau et al. 1988; Southwood 1988).

Local adaptations of plants and animals to long-term inundations have been investigated for several decades cooperatively between the National Institute for Amazonian Research (INPA) at Manaus/Brazil and the Tropical Ecology Group at the Max-Planck Institute for Limnology in Plön/Germany. The developed survival strategies in the Central Amazonian floodplains include phenological (life cycle), physiological, ethological and sporadically also morphological adaptations (Adis 1992b; Adis & Junk 2002). Furthermore, the resident terrestrial arthropods are divided into so-called 'migrants' and 'non-migrants'. The former seek refuge prior to the rising waters by seasonal migrations, i.e. predominantly by vertical ascents to the non-inundated trunk or canopy of trees, as well as by horizontal migrations to adjacent uplands (Adis 1997). In the latter case, several invertebrates stay near the water line and move according to the ascending or descending flood (Irmler 1979). The non-migrants, on the other hand, have acquired adaptations to endure the aquatic phase submersed (Irmler & Furch 1979; Scheller & Adis 1984; Adis 1992b), but long-term resistance to inundation is found in few species only (Adis 1981, 1986). As revealed by ecological studies, several terrestrial arthropod species that are plurivoltine in the non-flooded upland forests have secondarily immigrated to inundation forests and evolved a univoltine life cycle, i.e. they have only one generation per year (Adis et al. 1988; Adis 1997; Adis et al. 1997; Vohland & Adis 1999). This is due to the fact that the life cycle has to be timed with the seasonal pattern predetermined by the flood pulse. This pattern being an annual one, most species studied within the area are univoltine, i.e. they suspend reproduction during the aquatic phase and exclusively reproduce in the terrestrial phase.

The survival probability of terrestrial invertebrates in the floodplain areas depends on their 'ecological valence', i.e. on the diversity of their survival strategies (Adis 1997). The local life strategies may serve as an example for the relatively high ecological plasticity in millipedes (Hoffman et al. 2002). Most of the millipedes populating

inundation forests are terricolous and undertake temporary migrations up tree trunks to pass the aquatic phase (Adis 1992b; Adis et al. 1996a; Golovatch & Adis 1998). Following the retreating waters, such millipedes return to the ground for their reproduction that results in advanced developmental stages before the onset of the next inundation event. Other species, however, are arboreal and rarely visit the ground. The tree-dwelling millipede *Epinannolene exilio*, for instance, migrates to the lower trunk region only during strong insolation in the canopy region (Adis 1992b). In contrast, the only known semi-aquatic millipede from Amazonia, *Myrmecodesmus adisi*, can remain active as subadult instars underwater during the entire inundation period, being capable to resist submersion up to eleven months in the laboratory due to plastron structures (Adis 1986, 1992b; Messner & Adis 1988; Adis & Messner 1997). The mechanisms of adaptation and the survival strategies in Diplopoda are the more so intricate that each inundation forest type (Chapters 3.1.1.1-3) appears to support its own, often endemic, faunule, with only a few species sharing white-, black- and mixedwater floodplains, one of them being the eurytopic *P. obliterata* (Hoffman et al. 2002).

Like most other terrestrial invertebrates, *P. obliterata* escape seasonal flooding by vertical migrations to tree trunks (Adis 1986). So far, adult and subadult individuals but no juveniles have been found on stems during the aquatic phase (Adis, pers. commun.). The phenology of *P. obliterata* appears to resemble that of *Pycnotropis tida*, another resident polydesmidan millipede which passes inundation in the adult stage, aggregating above the water line (Vohland & Adis 1999). Decisive for survival during the aquatic phase appears to be the comparative dryness, since animals in the trunk region are exposed to high solar radiation and a relative humidity below 50 %. Juveniles are even more sensitive to these extreme environmental conditions than adults, i.e. to both submersion and desiccation (Vohland & Adis 1999). Given the vulnerability and, therefore, probably a low survival rate of immature stages, the lack of reproduction during this critical period might represent a reasonable local adaptation. However, survival of juvenile polydesmids in the trunk or canopy region is possible, as shown for the terricolous migrant *Cutervodesmus adisi* (Furhmannodesmidae) (Adis et al. 1996a).

1.4 Molecular Markers in Population Genetics

1.4.1 Definition of Molecular Markers

Molecular markers are small sections of the genome which represent single polymorphic loci consisting of either non-encoding or encoding sequences. These rather short DNA sequences are used as indicators of the genome-wide variation in organisms. Allozymes, the allelic variants of a single enzyme locus, were the first molecular markers to be applied widely in ecology. The level of variability at any individual marker locus ranges from one to tens or even hundreds of alternative alleles over a species' range. In population genetics, markers with a moderate level of polymorphism are most adequate because this generates larger sample sizes of each allele for statistical treatments (Beebee & Rowe 2004).

1.4.2 Subject of Population Genetics

Natural populations vary enormously in size, structure and dynamics. The size of populations can differ considerably, ranging from a dozen up to millions of specimens. Some populations are internally differentiated into clusters of individuals, i.e. subpopulations, more likely to breed with each other than with individuals from other subpopulations (Beebee & Rowe 2004). In 'metapopulations', which consist of multiple, geographically separated 'demes', the subpopulations are characterised by periodic local extinction and recolonisation (Hanski 1998). The degree to which a population is differentiated into demes depends on the connectivity between the respective biotope patches. At the scale of a species' total range, some extent of population subdivision is almost inevitable due to isolating geographical barriers. Furthermore, populations are also dynamic, i.e. changing in size and distribution over time. All of these properties may have genetic implications such as the genetic diversity within, or genetic differentiation between, populations. Consequently, by assessing these genetic effects, population genetics in turn addresses important aspects of population structure such as recent and historical population subdivision, dispersal, and size (Beebee & Rowe 2004).

1.4.3 Application of Molecular Markers

1.4.3.1 Classification and Speciation

Morphological characters are frequently used to identify individual species. However, given that several authors have discussed the plasticity of morphological traits (West-Eberhard 2003; DeWitt & Scheiner 2004); these features are an ambiguous criterion for the discrimination of subspecies or species. One of the main problems in species identification are 'cryptic' species complexes, i.e. biologically discrete species that are so similar in their morphology that their species distinctiveness is not recognised (Curčić et al. 1994). This dilemma is of particular importance for inconspicuous invertebrates (Wolf & Adis 1992). The failure to recognise such cryptic species, however, will invalidate any work on ecology, physiology and behaviour that is done on the assumption that only one species is involved (Ayala et al. 1975). Therefore, morphological characteristics alone are unsuitable for identifying ecological races, subspecies or species.

It has been assumed that body protein electrophoresis can be used to differentiate the taxonomic and evolutionary interrelations in both congeneric and other, more distant taxa (Curčić et al. 1994). The biological species concept (Mayr & Ashlock 1991) defines species as genetic units of naturally breeding populations which are reproductively isolated from other such groups. In this context, allozyme data provide a very powerful tool to test whether or not two taxa or forms share a common gene pool and thus belong to the same species (Hoess & Scholl 1999). The method also enables a genetic interpretation of ecological data and allows for a quantification of interspecific and intraspecific relationships to be made (Avise 1974; Ferguson 1980). In certain cases, such as sibling species, where morphological differentiation between species is almost impossible to trace, allozyme data can be used as convenient taxonomic characters, e.g. in *Daphnia* spec. (Wolf & Mort 1986). Another benefit of the technique is that it can be applied to any arthropod stage regardless of the morphological condition as long as the soluble proteins do not become denaturated. As a result, the use of biochemical genetic criteria in taxonomy has a long history (Wright 1974).

Indeed, this method has allowed clarifying taxonomic relationships in numerous insects (Ayala & Powell 1972; Fanciulli et al. 2000; Audisio et al. 2002), some arachnids (Vachon et al. 1972; May et al. 1977) as well as in the millipede group Glomeridae (Hoess & Scholl 1999). Studies of allozyme variation revealed several cryptic host-

associated species in insects (Nyman 2002; Nyman et al. 2002). In the same way, the classification of morphologically analogous *Glomeris*-taxa, *G. hexasticha* and *G. intermedia,* as separate species could be verified (Hoess et al. 1997), contrary to traditional taxonomy that treated *G. intermedia* as a subspecies of the former (Hoess & Scholl 1999). Species identity of geographically and morphologically separated bisexual and parthenogenetic populations of the millipede *Polyxenus lagarus* has also demonstrated using this technique (Duy-Jacquemin & d'Hondt 1998). Such evidence is noteworthy since problems with species delimitation are often caused by parthenogenesis which is common in most groups of invertebrates (Minelli & Foddai 1997). Furthermore, cryptic speciation in the *Anadenobolus excisus* millipede species complex, which is endemic to Jamaica and lacks discrete morphological differentiation, could only be revealed by molecular markers (Bond & Sierwald 2002). These sibling species have undergone speciation in the absence of the conspicuous divergence of male genitalia (Bond & Beamer 2003), a diagnostic character commonly used for species discrimination in millipedes (Minelli & Foddai 1997) (Chapter 1.2.1.1).

To summarise, molecular markers such as allozymes are qualified to study ecological speciation processes in the millipede *P. obliterata*.

1.4.3.2 Adaptive Divergence among Populations

Apart from estimating the genetic diversity of populations, polymorphic allozymes can be used to assess the population structure and the extent of migration between populations (Beebee & Rowe 2004). Recently, enzyme electrophoresis has been frequently been used to investigate the population genetics of small terrestrial invertebrates. Numerous studies on populations of individual species have been able to reveal spatial heterogeneity of genotype distribution, also for millipedes (Hoess & Scholl 2001). Allozyme data indicated the existence of three genetic provinces within the monomorphic cave millipede *Stygiochiropus communis* (Humphreys & Shear 1993), well in agreement with the geographical structure of the region (Humphreys & Adams 2001). Beyond these observations, some authors discovered correlations of allele frequencies with geographical or climatic clines, as described for populations of the millipede *Tetrarthrosoma syriacum* (Pavlíček & Nevo 1996). Such clines coincided with gradients of humidity or temperature in single cases (Mitton & Koehn 1975; Nevo et al. 1981). Even an association between allozyme frequencies and metal tolerance has

been observed for populations of a soil-dwelling insect (Frati et al. 1992) and ixodid ticks (Dubinina et al. 2004). Moreover, the genotype distribution of another terrestrial invertebrate has been shown to be correlated with dry and seasonally flooded forests in Central Amazonia (Wolf & Adis 1992). Therefore, distinct abiotic factors, such as periodic flooding, may be responsible for selective effects on the genotypic constitution of populations (cf. Anlauf & Neumann 1997). Whereas allozyme variability is expected to be sufficiently neutral and to evolve chiefly by genetic drift (Wang & Schreiber 1999; Hartl 1988; Kimura 1983), there are numerous examples, however, showing allozyme loci being under selection (Beebee & Rowe 2004), thereby reinforcing adaptive theories of molecular evolution (Gillespie 1978). A study of literature records across 400 to 1,111 species, representing diverse taxa from several phyla, reveals that protein diversity is generally positively correlated with broader geographic, climatic and biotope spectra (Nevo 1990, 2001). Allozyme diversity proves to be non-randomly distributed in nature, ecologically predictable, and largely processed, maintained, and oriented by natural selection. Therefore**,** genetic differentiation observed at apparently neutral markers can be used as indicator of adaptive divergence among populations (Morgan et al. 2001). This raises the question whether correlations exist between different biotope properties and the genetic constitution of natural populations of the eurytopic species *P. obliterata*.

1.4.4 Technique

Since 1955, protein electrophoresis using starch gel as the supporting medium separates protein variants according to their mobility in an electric field (Beebee & Rowe 2004). Due to their varying charges and masses, different proteins are divided into single fractions (Westermeier 2001). The examination of polymorphic enzyme loci (Hubby & Lewontin 1966) proved to be an appropriate tool in assessing the genetic differentiation between populations (Anlauf & Neumann 1997). The respective allelic variants of a single enzyme locus that differ in their electrophoretic mobility became known as allozymes.

Over the subsequent decades, hundreds of studies have used allozyme assays to address ecological and evolutionary questions (May 1992). The current method of choice in electrophoretic separation of enzymes is isoelectric focusing (IEF). The proteins move in a pH gradient towards the anode or cathode until they reach a position, where their

net charges are zero. This pH value is the 'isoelectric point' (pI) of the substance. The focusing effect yields sharp protein zones and a high resolution. Analytical focussing is carried out in either polyacrylamide or agarose gels. The benefits of agarose gels are faster separation and substantially larger pores, the gels components are not toxic and do not contain catalysts which could interfere with the separation (Westermeier 2001). During preliminary tests, agarose gels proved to be suitable for the analysis of allozyme variation in *P. obliterata* (pers. obs.). Allozyme electrophoresis has certain limitations for it underestimates protein variability and reveals presumably no more than 25 to 30 % of the 'true' level of amino acid substitution (Nei 1987).

Recently, new methods for population genetics based on polymerase chain reaction (PCR) have been introduced. In the PCR, small amounts of DNA are copied exponentially to generate large quantities, thereby allowing analyses based on tiny tissue fragments. Although these new techniques have the advantage of higher resolving power over allozymes, they also have disadvantages, i.e. dominance in AFLP markers (Vos et al. 1995), costs of developing and experience need in microsatellite library construction (Tautz 1989), and the current unresolved analytical problems in SNPs, being a new technique (Morin et al. 2004).

1.4.5 Appraisal: Choice of an Appropriate Marker

With allozymes and microsatellites being codominant markers, they are both suitable tools for investigating the genetic properties of populations (Beebee & Rowe 2004). In many cases, DNA markers revealed geographical structuring that was not paralleled by the variation at allozyme loci (e.g. Schaschl et al. 2003). The opposite has also been observed, because highly diverse loci such as microsatellites tend to underestimate between-population variance (Hedrick 1999). In a study of the Central Amazonian millipede *Pycnotropic tida*, populations from both flooded and non-flooded forest areas were genetically differentiated according to allozyme data, whereas microsatellites failed to discriminate the respective populations (Bachmann et al. 1998; Golovatch et al. 1998).

Considering the available resources and infrastructure at Manaus, AM, Brazil, enzyme electrophoresis was the most effective means to perform genetic analyses of single millipedes in the laboratory. Hence, intraspecific polymorphism in different populations of *P. obliterata* was recorded by isoelectric focussing of appropriate, scorable enzyme

systems, namely PGI, PGM, ME, GOT, PK and ACP (Table 18), that were chosen in preliminary tests using agarose gels as the supporting medium (Chapter 1.4.4).

2 THESIS OUTLINE

This thesis is divided into two chapters, considering comparative ecological as well as genetic aspects of different populations in the pyrgodesmidan millipede *Poratia obliterata* (Kraus, 1960). Both sections complement each other in a comprehensive examination of the adaptation processes in Central Amazonian populations of this eurytopic species.

Due to periodical high waters of the Amazon River, the adjacent forests are inundated for several months per year, leading to numerous adaptations in the terrestrial invertebrates inhabiting them. Their survival strategies in these seasonally flooded biotopes can be either based on ecological plasticity or on ecological speciation, i.e. 'biotope-specific races', subspecies or species. To elucidate the underlying process and get a better understanding of the adaptation events in this ecosystem, I used the small millipede *P. obliterata* as a model organism for terrestrial invertebrates. In the case of ecological differentiation, populations of the hypothesised ecotypes in non-flooded and seasonally inundated biotopes are expected to differ not only in terms of life history strategy but also in genetic structure.

This outline gives a short overview of the motivation for the different investigations.

2.1 Part I: Ecological Traits

In the first chapter, the life history and behaviour of *P. obliterata* populations from non-flooded upland and various seasonally flooded biotopes (white-, black- and mixedwater inundation forests) in Central Amazonia were compared. Field studies were complemented with explorative experiments concerning migration and reproduction.

To reveal ecological, biotope-specific characteristics in the populations, I examined the morphology and monitored seasonal migration, microhabitat preference, gregarious behaviour, interaction with other millipede species and phenology during the aquatic phases in 2002 and 2003. In addition, I measured relevant abiotic factors in the field such as the duration of flood periods and the humidity in microhabitats. Observations showed that animals in inundation forests escape flooding by a vertical migration to tree trunks. To explore the potential for such a migration in response to rising waters, I performed flooding experiments with individuals from both non-flooded and seasonally inundated areas. Since field studies showed distinct life cycles in the respective

populations, I also conducted experiments to detect a possible factor that regulates reproduction. The factors considered to affect both fertility and offspring in *P. obliterata* were habitat substrate, mean air temperature, relative humidity and nutrition. All results are discussed in the context of life history strategies that are adaptive to the respective biotopes.

2.2 Part II: Genetic Variation

The second chapter addresses whether the observed adaptive ecological traits involve ecological speciation processes. Allozyme data provide an effective tool not only to assess cryptic speciation but also adaptive divergence among populations in diverse biotopes. Therefore, I employed allozyme diagnostics in order to reveal effects of the different biotope types on the genetic constitution of local populations in *P. obliterata*. Since both biotope quality and geographic distance influence the genetic differentiation between subpopulations, spatial connectivity and migration were also considered. The geographical and ecotype specific patterns of allozyme variation in ten subpopulations from Central Amazonia and one subpopulation from Amapá State, eastern Amazonia are described. Localities were chosen in relation to four different biotopes, i.e. non-flooded plantations versus diverse seasonally inundated forests, and the course of the Solimões and Negro rivers, both considered potential dispersal pathways. I examined genetic diversity and heterozygosity within as well as variability among subpopulations of *P. obliterata* and evaluated the correlation between genetic and geographic distance. The extent to which the observed genetic patterns could be explained in terms of colonisation hypotheses and local versus general adaptation strategies are discussed.

3 ECOLOGICAL TRAITS

3.1 Material and Methods

3.1.1 Study Sites

3.1.1.1 Várzea

The whitewater or Várzea study site was situated on Marchantaria Island (3°15' S, 59°58' W), the first island in the lower Solimões River (Fig. 2), approximately 15 km upstream of the confluence with the Negro River (Klinge et al. 1995). It has been used by the INPA/Max-Planck research group since 1980 to investigate the local terrestrial invertebrate fauna and their ecology (cf. Junk 1997). The island consists of alluvial sediments, predominantly montmorillonite, and is approximately 5,000 years in age. The main sampling site, located at the upper Lake Camaleão, is inundated for an average of 5.6 ± 1.5 months per year (n = 95 years), with the water leveling to a height of approximately 5.5 m (Adis 2002).

P. obliterata was collected on the northern bank of Lake Camaleão, where 15 trees along a transect of 1.85 km were sampled. The beginning and end were marked by sample tree 1 and 13 respectively (Fig. 6; Table 1). Only one additional sample tree (n° 15) was located at the southern lakeside, with a linear distance of 0.6 km to tree 13.

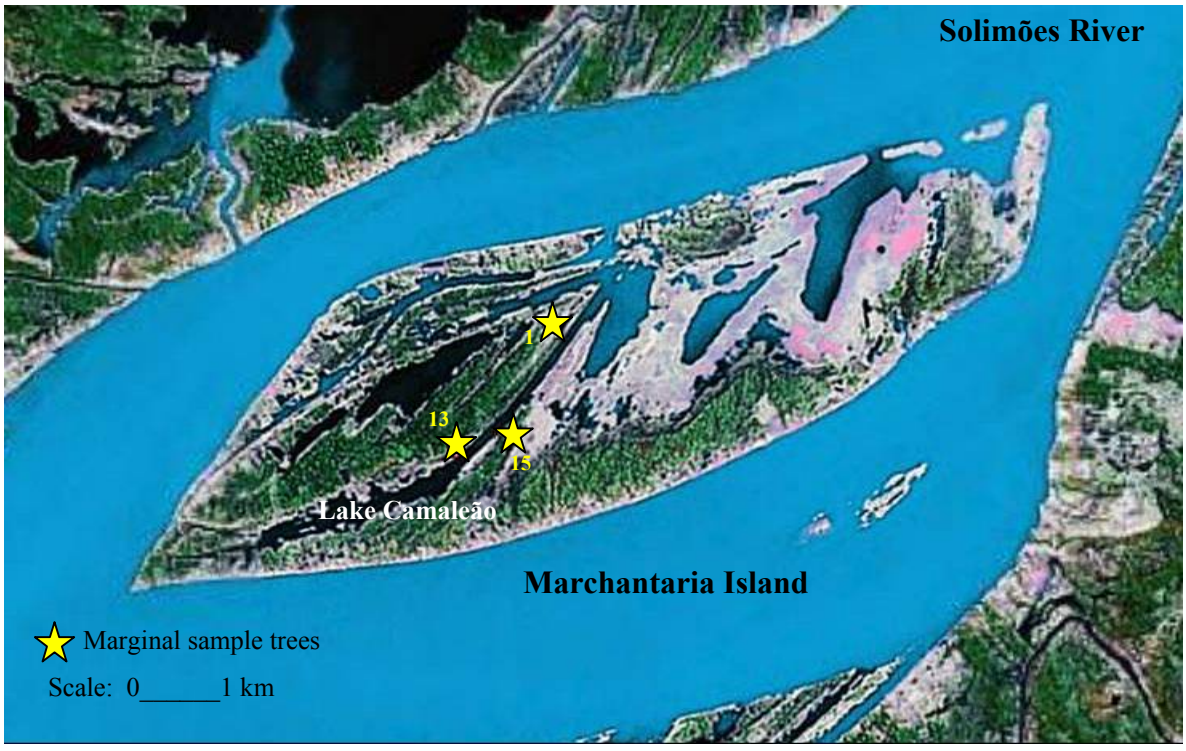

Figure 6. Marginal sample trees at the study site in the whitewater inundation forest at Lake Camaleão, Marchantaria Island (3°15' S, 59°58' W) in the Solimões River (Picture: Google Earth). Length of transect between tree 1 and tree 13: 1.8 km. Linear distance between tree 13 and tree 15: 0.6 km.

Table 1. Trees sampled for *P. obliterata* at Marchantaria Island. Identification number (N°), tree species, position and minimum flood period in 2002 and 2003 (Chapter 3.1.3) are listed. Abbreviation: d, days.

N°	Tree species	GPS-data	Duration of minimum flood period
01	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°14.49 S 059°57.12 W	202 d; (07.03.-24.09.02) 174 d; (30.03.-19.09.03)
02	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°14.49 S 059°57.12 W	202 d; (07.03.-24.09.02) 174 d; (30.03.-19.09.03)
03	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°14.49 S 059°57.12 W	198 d; (10.03.-23.09.02) 171 d; (01.04.-18.09.03)
04	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°14.53 S 059°57.15 W	198 d; (10.03.-23.09.02) 171 d; (01.04.-18.09.03)
05	*Nectandra amazonum* Nees, Lauraceae	03°14.65 S 059°57.25 W	198 d; (10.03.-23.09.02) 171 d; (01.04.-18.09.03)
06	*Nectandra amazonum* Nees, Lauraceae	03°14.65 S 059°57.25 W	198 d; (10.03.-23.09.02) 171 d; (01.04.-18.09.03)
07	*Eschweilera ovalifolia* [DC.] Nied., Lecythidaceae	03°14.72 S 059°57.30 W	198 d; (10.03.-23.09.02) 171 d; (01.04.-18.09.03)
08	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°14.74 S 059°57.31 W	174 d; (26.03.-15.09.02) 150 d; (15.04.-11.09.03)
09	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°14.83 S 059°57.39 W	198 d; (10.03.-23.09.02) 171 d; (01.04.-18.09.03)
10	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°14.88 S 059°57.42 W	198 d; (10.03.-23.09.02) 171 d; (01.04.-18.09.03)
11	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°14.72 S 059°57.28 W	198 d; (10.03.-23.09.02) 171 d; 01.04.-18.09.03
12	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°14.88 S 059°57.42 W	202 d; (07.03.-24.09.02) 174 d; (30.03.-19.09.03)
13	*Eschweilera ovalifolia* [DC.] Nied., Lecythidaceae	03°15.28 S 059°57.83 W	202 d; (07.03.-24.09.02) 174 d; 30.03.-19.09.03
14	*Vitex cymosa* Bertero ex Spreng., Verbenaceae	03°15.28 S 059°57.82 W	218 d; (22.02.-28.09.02) 190 d; 18.03.-23.09.03
15	*Vitex cymosa* Bertero ex Spreng., Verbenaceae	03°15.16 S 059°57.52 W (other lakeside)	218 d; (22.02.-28.09.02) 190 d; (18.03.-23.09.03)
16	*Eschweilera ovalifolia* [DC.] Nied., Lecythidaceae	03°14.72 S 059°57.31 W	198 d; (10.03.-23.09.02) 171 d; (01.04.-18.09.03)

In the local inundation forest (Fig. 7), 47 arboreal species representing 46 genera in 25 families have been recorded. The most abundant and common species are *Crataeva benthamii* (Capparidaceae), *Laetia corymbulosa* (Flacourtiaceae) and *Vitex cymosa* (Verbenaceae) as well as *Eschweileria* sp. (Lecythidaceae), *Pterocarpus amazonum* (Fabaceae), *Calophyllum brasiliense* (Clusiaceae) and *Bactris* sp. (Aracaceae), all of which are known to be typical floristic elements of the seasonal Várzea (Klinge et al.

1995; Worbes 1997). Native trees reach some 28 m in height and carry only few epiphytes. The upper canopy of the forest, which is relatively open, is dominated by *Pseudobombax munguba* (Bombaceae), which covers the highest basal area (Klinge et al. 1995; Adis 2002).

Figure 7. Seasonally flooded whitewater inundation forest (Várzea) at Marchantaria Island (3°15' S, 59°58' W), Amazonia State, Brazil (Picture: N.G.R. Bergholz).

The majority of terrestrial arthropods in this Várzea island forest (Acari and Collembola excluded) were found to live in the trunk/canopy region (Adis & Schubart 1984). In the canopy, adult Coleoptera (21 %), Formicidae (20 %) and adult Diptera (13 %) represented the main fraction of the total number of arthropods obtained by pyrethrum-fogging after the flood had receded from the forests. The dominance of groups, however, varied with the sampled tree species. The trunk fauna was dominated by Collembola and Acari, which accounted for over 95 % of the total Arthropoda. During the terrestrial phase, i.e. emersion period, numerous animals came down to the forest floor for feeding (especially Formicidae) or oviposition (particularly adult Coleoptera and Diptera). Araneae (23 % of the total catch) were also numerous. On the forest floor, Acari (49 to 58 %) and Collembola (11 to 24 %) dominated in the litter and upper soil layer. Excluding these two taxa, Formicidae (32 %) and Diplopoda (22 %, mainly Polydesmida) were the most frequent arthropods. Conditions for a rich soil fauna were

considered unfavorable due to the very thin litter layer of Várzea forests, which results from leaves being either adhered due to annually deposited river sediments or lost during inundation by the strong current (Adis 1997).

More information concerning the area, its resident flora and inhabiting terrestrial invertebrates is available in the literature (Irion et al. 1983; Adis 1992b; Klinge et al. 1995; Junk 1997).

3.1.1.2 Igapó

In the blackwater area, the study site was situated at the lower course of the Tarumã Mirím River (3°02' S, 60°17' W), a tributary of the Negro River approximately 20 km upstream of Manaus (Fig. 2). The site has also frequently been used for studies on terrestrial invertebrates by the INPA/Max-Planck research group. The local blackwater forest or seasonal Igapó (Fig. 8) is situated on a slope and extends from the non-inundated upland area (Terra firme), which is covered by secondary forest (cut but unburned 'Capoeira'), with a constant decline (< 5 %) toward a sandy shoreline (Adis et al. 1996a). The central part of the study site, which is located in upper Igapó (cf. Adis 1984), is covered for an average of 3.9 ± 1.5 months per year (n = 95 years) by up to 4 m of floodwater (Adis 2002).

Figure 8. Seasonally flooded blackwater inundation forest (Igapó) at the Tarumã Mirím River (3°02' S, 60°17' W), Amazonia State, Brazil (Picture: N.G.R. Bergholz).

Since *P. obliterata* were less common on this more heterogeneous site, animals were not collected along a transect but in areas at four different sublocations (Fig. 9; Table 2). The northernmost place comprised solely one sample tree (n° 2), whereas two trees (n° 16 and 3, linear distance in-between: 0.04 km) were sampled at the nearest southern position in approximately 3 km distance. The close-by third location (in 0.6 km distance, marked by tree 13) covered an area of 0.2x0.2 km, including four sample trees (n° 4, 11 to 13 and 15). The last site was located 2.8 km further to the southeast (marked by tree 14); it amounted to an area of 0.4x0.1 km, comprising five sample trees (n° 5 to 6, 8 to 10 and 14).

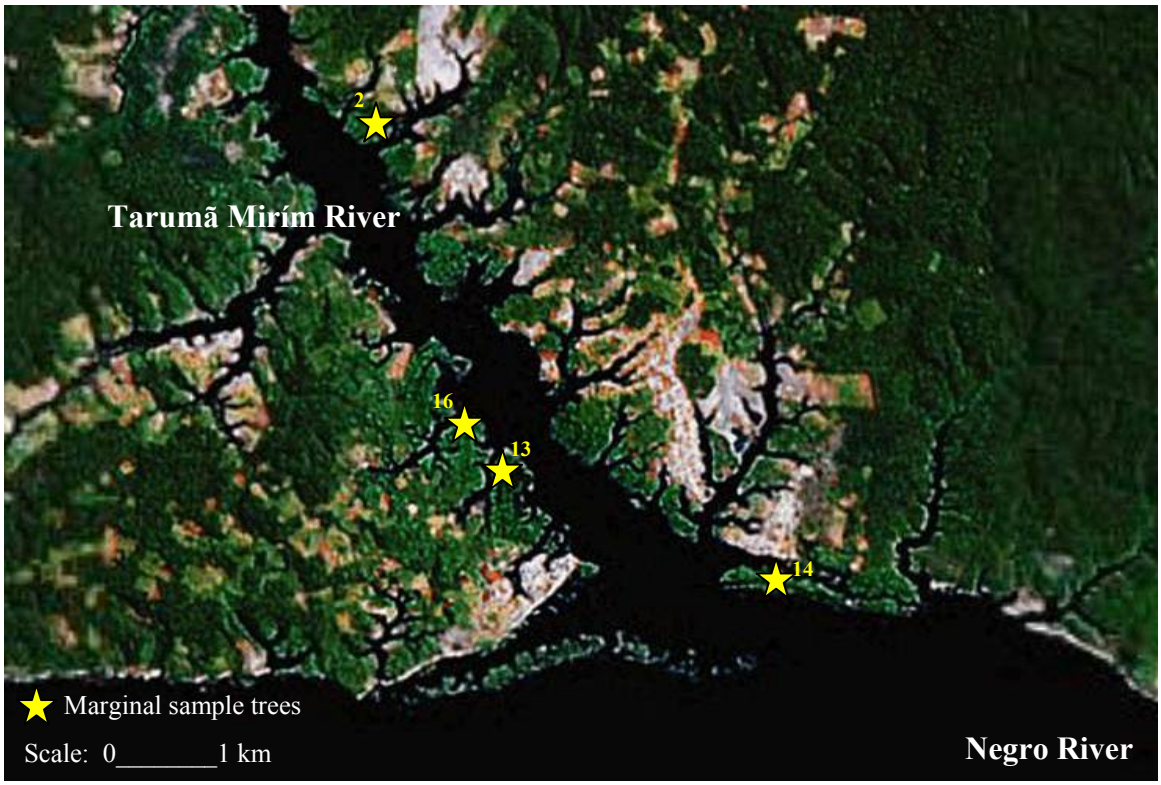

Figure 9. Marginal sample trees at the study site in the blackwater inundation forests at Tarumã Mirím River (3°02' S, 60°17' W), a tributary of the Negro River (Picture: Google Earth). Linear distance between tree 13 and trees 16, 14 and 2, respectively: 0.6 km, 2.8 km and 3.6 km.

In the upper Igapó, 48 arboreal species have been recorded, with the trees reaching 35 m in height and *Aldina latifolia* (Fabaceae) representing the dominant species. The canopy is almost closed and colonised by numerous epiphytes (Adis 1984, 2002). The most abundant species are *Virola elongata* (Myristicaceae), *Eschweileria longipes* and *E. pachysepala* (Lecythidaceae) and *Pithecellobium amplissimum* (Mimosaceae) (Adis 1984).

Table 2. Trees sampled for *P. obliterata* at Tarumã Mirím River. Identification number (N°), tree species, position and minimum flood period in 2002 and 2003 (Chapter 3.1.3) are listed. Abbreviation: d, days.

N°	Tree species	GPS-data	Duration of minimum flood period
02	*Calophyllum brasiliense* Camb., Clusiaceae	02°59.35 S 060°11.58 W subsite 1	195 d; (12.03.-22.09.02) 169 d; (03.04.-18.09.03)
03	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°00.91 S 060°11.10 W subsite 2	183 d; (21.03.-19.09.02) 157 d; (11.04.-14.09.03)
04	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°01.07 S 060°10.92 W subsite 2	171 d; (28.03.-14.09.02) 149 d; (16.04.-11.09.03)
05	*Tabebuia barbata* [E. Mey] Sandw., Bignoniaceae	03°01.70 S 060°09.66 W subsite 3	195 d; (12.03.-22.09.02) 169 d; (03.04.-18.09.03)
06	*Tabebuia barbata* [E. Mey] Sandw., Bignoniaceae	03°01.69 S 060°09.66 W subsite 3	195 d; (12.03.-22.09.02) 169 d; (03.04.-18.09.03)
08	*Tabebuia barbata* [E. Mey] Sandw., Bignoniaceae	03°01.69 S 060°09.65 W subsite 3	195 d; (12.03.-22.09.02) 169 d; (03.04.-18.09.03)
09	Not identified	03°01.67 S 060°09.53 W subsite 3	142 d; (15.04.-03.09.02) 119 d; (03.05.-29.08.03)
10	*Aldina latifolia* Spruce ex Benth., Fabaceae	03°01.68 S 060°09.53 W subsite 3	134 d; (20.04.-31.08.02) 112 d; (07.05.-26.08.03)
11	Not identified	03°01.10 S 060°10.89 W subsite 2	183 d; (21.03.-19.09.02) 157 d; (11.04.-14.09.03)
12	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°01.16 S 060°10.89 W subsite 2	183 d; (21.03.-19.09.02) 157 d; (11.04.-14.09.03)
13	Not identified	03°01.17 S 060°10.90 W subsite 2	183 d; (21.03.-19.09.02) 157 d; (11.04.-14.09.03)
14	*Aldina latifolia* Spruce ex Benth., Fabaceae dead wood, upright	03°01.71 S 060°09.46 W subsite 3	134 d; (20.04.-31.08.02) 112 d; (07.05.-26.08.03)
15	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°01.08 S 060°10.85 W subsite 2	195 d; (12.03.-22.09.02) 169 d; (03.04.-18.09.03)
16	Not identified	03°00.89 S 060°11.09 W subsite 2	134 d; (20.04.-31.08.02) 112 d; (07.05.-26.08.03)

The soil consists of clay, silt and sand material with alternating fractions and carries an organic layer of approximately 5 to 10 cm thickness. Its fine humus is penetrated by a

matting of roots rich in terricolous invertebrates (Adis 1977) and supports up to 3 cm of leaf litter (Adis et al. 1997).

The arthropod community in the canopy of Igapó forests resembles that of Várzea woodlands (see above): ants (43 %) dominated and, together with adult Diptera (15 %) and beetles (5 %), they represented more than half of the total catch obtained by pyrethrum fogging (Adis et al. 1984). However, most of these inhabitants were represented by different species compared to the canopy guild in the Várzea (Adis 1997). Furthermore, the respective arthropod density in the Igapó is probably twice as high as that found in the Várzea, although still less than half of that given in primary upland forests (Adis et al. 1984). As for the trunk fauna, Collembola dominated but their catch numbers were far lower compared to those in the Várzea. The activity densities of trunk-dwelling arthropods other than Collembola and Acari were similar to those obtained in the Várzea. However, trunk ascents and descents in the Igapó were characterised by 'migrating terricolous' animals, which passed the aquatic phase in the arboreal region, i.e. Araneae, Pseudoscorpiones, Diplopoda, Symphyla and Chilopoda. In the litter and upper soil layer, Acari and also Collembola again dominated (Adis 1997). Apart of these two groups, non-flying terricolous migrants were the most abundant arthropods. However, in the lower Igapó, where the aquatic phase lasted longer than seven months per year on average, these migrants were absent (Adis 1997).

This seasonal forest has been periodically flooded for at least one million years (Adis 2002). More information concerning the region, its vegetation and terrestrial invertebrates can be found in the literature (Adis 1981, 1984; Worbes 1983; Junk 1997).

3.1.1.3 Várzea & Igapó

The mixedwater or seasonal Várzea & Igapó study site was situated at the Lake Janauarí (3°20' S, 60°17' W), located on a strip of land between the Negro and Solimões rivers, approximately 10 km distant from Manaus (Fig. 2). The region is influenced by blackwater of the Negro River during low water level and by whitewater of the Solimões River during the high water period. It is part of the Ecological Park of SELVATUR, Manaus, and a study area of the INPA/Max-Planck research group for investigations of the terrestrial invertebrate fauna (Amaral et al. 1997). The site is relatively flat and has no direct connection to the non-flooded upland areas several km away (Adis et al. 1997). The soil consists of clay, predominantly montmorillonite,

which represents alluvial deposits of the Solimões River (Adis 2002). The area is situated approximately 26.3 m above sea-level (Amaral et al. 1997) and is inundated for an average of 3.1 ± 1.6 months per year (n = 95 years), with the water leveling to a height of approximately 3 m (Adis 2002).

P. obliterata was sampled along a transect of 0.7 km (marked by trees 3 and 18), delimited by a creek flowing through the mixedwater inundation forest (Fig. 10; Table 3). The transect encompassed 15 sample trees (n° 1 to 2, 5 to 8, 10 to 12 and 14 to 18). In addition, *P. obliterata* were taken from two sample trees located 1.6 km further south (n° 9 and 13, linear distance in-between: 0.1 km).

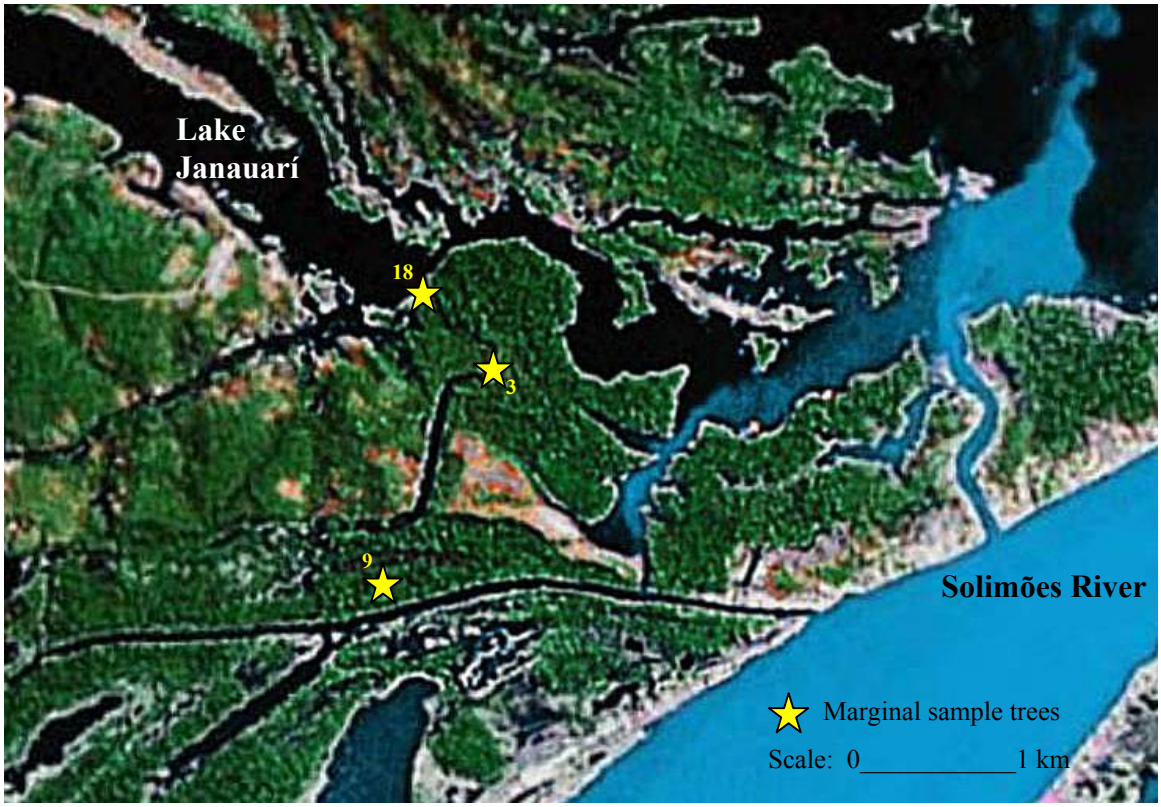

Figure 10. Marginal sample trees at the study site in the mixedwater inundation forest, Lake Janauarí (3°20' S, 60°17' W), between the Negro and Solimões River (Picture: Google Earth). Length of transect between tree 3 and tree 18: 0.7 km. Linear distance between tree 3 and tree 9: 1.6 km.

The study site (Fig. 11) represented an ecotone, i.e. the flora and terrestrial invertebrates of both Várzea and Igapó as well as occasional faunal representatives of non-flooded forests, with predominantly white- and some blackwater specific tree species (Adis & Schubart 1984; Adis et al. 1996b; Amaral et al. 1997; Morais et al. 1997a, 1997b). The most abundant species were *Poutera* sp. (Sapotaceae) and *Pterocarpum amazonum* (Fabaceae), whereas the highest basal area was covered by *Calophyllum brasiliense* (Clusiaceae). In total, 66 arboreal species representing 55 genera and 31 families were

recorded (Amaral et al. 1997). Similar to the whitewater study site (Marchantaria Island, see above), only a minor litter layer is formed during the terrestrial phase. It is mostly removed from the forests by the current of annual floodwaters and/or partially covered by sediments deposited during inundation (Adis et al. 1997).

Table 3. Trees sampled for *P. obliterata* at Lake Janauarí. Identification number (N°), tree species, position and minimum flood period in 2002 and 2003 (Chapter 3.1.3) are listed. Abbreviation: d, days.

N°	Tree species	GPS-data	Duration of minimum flood period
01	Not identified	03°13.09 S 060°01.23 W	188 d; (17.03.-20.09.02) 163 d; (07.04.-16.09.03)
02	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°13.08 S 060°01.26 W	174 d; (26.03.-15.09.02) 150 d; (15.04.-11.09.03)
03	*Eschweilera ovalifolia* [DC.] Nied., Lecythidaceae	03°13.30 S 060°01.06 W	135 d; (19.04.-31.08.02) 112 d; (07.05.-26.08.03)
05	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°13.20 S 060°01.08 W	188 d; (17.03.-20.09.02) 163 d; (07.04.-16.09.03)
06	Not identified	03°13.20 S 060°01.08 W	188 d; (17.03.-20.09.02) 163 d; (07.04.-16.09.03)
07	*Cassia leiandra* Benth., Fabaceae	03°13.26 S 060°01.07 W	218 d; (22.02.-28.09.02) 190 d; (18.03.-23.09.03)
08	*Calophyllum brasiliense* Camb., Clusiaceae dead wood, upright	03°13.28 S 060°01.06 W	119 d; (28.04.-24.08.02) 96 d; (16.05.-18.08.03)
09	*Eschweilera ovalifolia* [DC.] Nied., Lecythidaceae	03°14.07 S 060°01.44 W	144 d; (14.04.-04.09.02) 122 d; (02.05.-31.08.03)
10	*Eschweilera ovalifolia* [DC.] Nied., Lecythidaceae dead wood, upright	03°13.12 S 060°01.23 W	188 d; (17.03.-20.09.02) 163 d; (07.04.-16.09.03)
11	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°13.15 S 060°01.16 W	157 d; (06.04.-09.09.02) 135 d; (24.04.-05.09.03)
12	Not identified dead wood, upright	03°13.13 S 060°01.20 W	198 d; (10.03.-23.09.02) 171 d; (01.04.-18.09.03)
13	*Eschweilera ovalifolia* [DC.] Nied., Lecythidaceae	03°14.02 S 060°01.48 W	144 d; (14.04.-04.09.02) 122 d; (02.05.-31.08.03)
14	*Eschweilera ovalifolia* [DC.] Nied., Lecythidaceae	03°13.06 S 060°01.27 W	218 d; (22.02.-28.09.02) 190 d; (18.03.-23.09.03)
15	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°13.07 S 060°01.26 W	198 d; (10.03.-23.09.02) 171 d; (01.04.-18.09.03)
16	*Macrolobium acaciifolium* [Benth.] Benth., Fabaceae	03°13.09 S 060°01.24 W	188 d; (17.03.-20.09.02) 163 d; (07.04.-16.09.03)
17	Not identified	03°13.12 S 060°01.21 W	135 d; (19.04.-31.08.02) 112 d; (07.05.-26.08.03)
18	*Eschweilera ovalifolia* [DC.] Nied., Lecythidaceae	03°13.04 S 060°01.30 W	174 d; (26.03.-15.09.02) 150 d; (15.04.-11.09.03)

Further information concerning the study site is given by Adis (1992) and other authors (Morais 1995; Irmler 1975).

Figure 11. Seasonally flooded mixedwater inundation forest (Várzea & Igapó) at the Lake Janauarí (3°20' S, 60°17' W), Amazonia State, Brazil (Picture: N.G.R. Bergholz).

3.1.1.4 Terra firme

3.1.1.4.1 Banana Plantation at CPPA/Embrapa

The upland or Terra firme study site was a banana plantation (2°53´ S, 59°59´ W) situated on the cultivated area of the 'Agroforestry Research Station of the Western Amazon', i.e. CPPA (Centro de Pesquisa Agroflorestal da Amazônia Ocidental), Embrapa (Fig. 2), at km 30 on the Manaus-Itacoatiara highway (AM-010) near Manaus. Specimens of *P. obliterata* were first collected (October 1997, leg. J. Adis) in the litter composed of hollow palm boles on a peach palm tree plantation of *Bactris gasipaes* Kunth (Araceae) (Adis et al. 2001). The seeds of the cultivated palms were imported approximately 20 years ago from Peru (Garcia, pers. commun). Since the introduced seeds were delivered together with moistened substrate, e.g. sawdust (Garcia, pers. commun.), they possibly also contained concealed millipedes which established the respective population.

During my own investigations, *P. obliterata* could not be found on the relevant palm tree plantation, but was abundant on the adjacent, frequently fertilised banana (*Musa* sp.; Musaceae) plantation. This banana plantation (cf. Fig. 12) was established in January 1999 and consisted of plants introduced as seedlings, mostly transported without substrate, from Bahia State, eastern coast of Brazil (Garcia, pers. commun.). Specimens of *P. obliterata* were collected at 24 different sample sites scattered randomly across an area of 0.17x0.14 km.

Figure 12. Banana (*Musa* sp.; Musaceae) plantation (Picture: www.junglemedicmissions.org).

3.1.1.4.2 Upland Forest Reserve 'Reserva A. Ducke'

The upland forest reserve 'Reserva Florestal Adolpho Ducke' ('Reserva A. Ducke') is one of the best studied areas of Amazonian rainforest and part of the INPA (National Institute for Amazonian Research) at Manaus, Brazil. It is covered by 90 km^2 of mostly undisturbed rainforest (Fig. 13) and situated 26 km northeast of the city (2°54´ S, 59°58´ W; Fig. 2) (Adis 2002). A description of local geology, soil characteristics, flora and fauna is available in the literature (Penny & Arias 1982; Adis et al. 1984; Gentry 1990; Adis et al. 2002).

Figure 13. Primary upland rainforest at 'Reserva A. Ducke' (2°54´ S, 59°58´ W), Amazonia State, Brazil (Picture: N.G.R. Bergholz).

The millipede *P. obliterata* has been recorded at the site (Adis et al. 2002), but due to its rarity in this biotope (Golovatch, pers. commun.) could not be found during my own repeated searches. Only few specimens of other small polydesmidan millipede species were encountered in fallen dead wood on the ground (*Cutervodesmus* sp., Fuhrmannodesmidae; *Docodesmus* sp., Pyrgodesmidae).

3.1.2 Monitoring and Sampling of *P. obliterata* in the Field

3.1.2.1 Inundation Forests

During the rainy season (Chapter 1.1.2), many soil arthropods can be found in the upper soil, whereas in the dry season they inhabit the lower layers and are hard to locate during the terrestrial phase (Adis, pers. commun.). Like most terricolous millipedes that live in the forests' humus layer, the species *P. obliterata* avoids flooding by retreating to the non-inundated trunk area (Adis 1981, 1986). Therefore, individuals can easily be collected during the aquatic phase. The species can be identified, as adults are found on

tree trunks during this time of the year (Adis, pers. commun.). In Diplopoda, male gonopod structures are required for an accurate taxonomic determination (Chapter 1.2.1.3).

The study sites at Marchantaria Island, Tarumã Mirím River and Lake Janauarí were regularly visited during the aquatic phase, i.e. from March/April to September/October, in 2002 and 2003. In the first year, fortnightly collections were conducted to monitor the life cycle in the field, whereas in the second year, animals were collected at least monthly. To observe the migration behaviour, the sites were observed at fortnightly intervals at both the onset and end of the aquatic phases in the two years. Trees at the study sites were also surveyed at low water levels to detect the time of trunk ascents and descents as well as the local duration of flooding.

During the aquatic phase, the sampling sites within the different inundation forests were reached by canoe. At least 14 sample trees per study site were chosen for permanent monitoring on the basis of repeated findings of *P. obliterata*. The respective trees were numbered at the first collection, labeled, characterised by means of GPS data as well as individual landmarks and, mostly, identified to species level. The trees were regularly visited to observe the progress of developmental stages and generations of *P. obliterata* in the field.

The number of specimens sampled depended on availability. Generally, all encountered millipedes were collected unless densities were rather high. To trace individuals, several bark pieces of living trees were removed up to 1 m above the water level. Subsequently, the back of the removed bark and the bare trunk region were searched for small and cryptic millipedes. In addition, the decomposing wood inside floating or inundated dead tree trunks was examined for specimens. To avoid damage, the delicate animals were collected by means of a fine, moistened brush. They were stored in small labeled boxes containing moist earth substrate and transported to the laboratory, where age and sex were determined. After classification, the respective animals were kept both for rearing and experiments on vertical migration and reproduction under several conditions (Chapter 3.1.6). Some of the adult females sampled in August and September 2003 were dissected and screened for eggs.

To characterise preferred bark microhabitats and the observed gregarious behaviour, the number of specimens per aggregate and their position relative to the water line were recorded for the individuals sampled and for those merely observed during regular visits. Aggregates or groups were defined as a quantity of individuals clustered together,

mostly with tight body contact. The presence of other small millipede species in the aggregates was noted and some individuals were sampled for identification. To verify if *P. obliterata* also take refuge in the moist root substrate or leaf cisterns of remote epiphytes, which grow mostly in the upper trunk region, several epiphytes (in 2.0 to 7.5 m height) were sampled at Tarumã Mirím River in August 2002.

3.1.2.2 Banana Plantation

On the upland study site, the phenology of *P. obliterata* was monitored parallel to the time schedule performed in the inundation forests, i.e. from March/April to September/October, in 2002 and 2003. In 2002, fortnightly collections were made, while in 2003 the site was visited at least every month.

A total of 24 different sampling sites, scattered across the field, were chosen by random encounters of *P. obliterata*. The respective sub-sites were marked by numbers as well as GPS data. Due to the agricultural management, some cut banana stems were left to decompose on the plantation among the living banana plants. The very moist plant parts were slit open and inspected for *P. obliterata*. If specimens were encountered, parts of the respective plant material were bagged and transported to the laboratory. Here, the decaying substrate was carefully stripped down to separate animals with a fine brush. Thereafter, the age and sex of the respective individuals which were then kept for rearing and experiments (Chapter 3.1.6) were determined. The presence of other small millipede species was also recorded and some individuals were sampled for identification.

3.1.3 Estimation of Flood Periods for Individual Sample Trees

In 2002, the inundation forests could only be visited when the aquatic phase had already started. Individual sample trees in the areas were monitored until October, when the forest had already fallen dry again. In 2003, observations started at the end of the terrestrial phase and were conducted during the subsequent aquatic phase until the next terrestrial phase. In considering the water level of the Negro River on the first (2003) and last collecting dates (2002 and 2003), when individual trees were found flooded, a threshold level for inundation could be estimated for each tree. Using this threshold value as a reference, the minimum flood period (based on fortnightly observations, the

exact period cannot be given) for single trees and thus for the average sampling areas could be calculated for both years.

3.1.4 Determination of Humidity in Microhabitats

To compare local humidity conditions for millipedes on the banana plantation and in the inundated forests, the water content of the respective habitat substrates was quantified. During the aquatic phase, ten trees colonised by *P. obliterata* were selected in the inundated forest of Marchantaria Island. Each 30 bark pieces were collected at different heights above the water line (at 0 to10, 20 and 50 cm distance) from the sunny and shady side of trees. On the banana plantation, 30 samples of various decaying banana stems inhabited by *P. obliterata* were taken. In the laboratory, all samples were weighed instantly to determine their fresh weight and subsequently kept for approximately three weeks in a desiccator until dry weight could be assessed.

3.1.5 Identification of Collected Millipedes

Species identification of living *P. obliterata* based on distinctive morphological characters in adults such as gonopods in males, body size and colour as well as shape and number of segments and appendages (Figs 4 & 14; Chapter 1.2.1.3). For this purpose, the fragile and heliophobous specimens were cautiously inspected using a fine brush (00 in thickness) and a stereo microscope (10 to 40-fold), with illumination adjusted as low as possible. In addition, some adult, predominantly male specimens from each locality were preserved in alcohol to serve as a taxonomic reference. In these individuals, the preparation and microscopic examination of male gonopods reconfirmed previous species identifications (cf. Adis et al. 2001). Specimens of other small millipede species were likewise identified, and preserved in alcohol as taxonomic vouchers.

The determination of sex and developmental stage in the sampled *P. obliterata* were mainly based on external characters. Mature males are distinguished by the presence of their elaborate gonopods, a modified pair of legs on the seventh body segment (Fig. 14).

Figure 14. *Poratia obliterata*: the gonopods (i.e. genitalia) of an adult male (size: 6 mm), situated at the seventh body segment (Picture: N.G.R. Bergholz).

From the beginning of the fourth developmental stage, immature males are characterised by the absence of a leg pair on the respective segment, but the genitalic primordia are barely visible. Immature females can easily be distinguished from mature individuals, since the maturity in *P. obliterata* is associated with the number of body segments which ranges from seven in the first developmental stage up to 20 in adults (Table 4; Chapter 1.2.1.4). The coloration of individuals also changes during development. Immatures are pale whitish during the first four developmental stages, but become darker from the fifth stage onwards until they obtain the characteristic brown-pinkish colour in the adult stage.

Table 4. Morphological characteristics of developmental stages in *P. obliterata*. The number of developmental stages along with the stage of maturation, the number of body segments and the number of leg pairs in both females and males are listed (Adis, pers. commun.; cf. Schubart 1934).

N° of developmental stage	Stage of maturation	N° of body segments (with legs)	N° of leg pairs (doubled leg pairs)	
			female	male
1	juvenile	7 (3)	3	3
2	juvenile	9 (5)	6 (1)	6 (1)
3	juvenile	12 (7)	11 (4)	11 (4)
4	juvenile	15 (10)	17 (7)	16 (6)
5	juvenile	17 (13)	23 (10)	22 (9)
6	juvenile	18 (15)	27 (12)	26 (11)
7	subadult	19 (16)	29 (13)	28 (12)
8	adult	20 (17)	31 (14)	30 (13)

3.1.6 Laboratory Experiments

3.1.6.1 Flooding Experiment

To compare the potential response to rising waters by vertical migration, specimens from seasonally inundated and non-flooded upland areas were monitored in a flooding experiment. To simulate a tropical forest microhabitat, the bottom of two plastic boxes (15x8 cm base area, 16x9 cm top area, 12 cm height) was covered with clay brought from the field (approx. 1 cm in height), earth substrate (approx. 0.5 cm in height; for composition see Chapter 3.1.6.2) and a thin top layer of native leave, all well moistened. To imitate tree trunks, a large piece of bark (c.a. 6x12 cm; *Macrolobium acaciifolium*, *Eschweilera ovalifolia*: see Chapter 3.1.6.2) was positioned at the broad side of each box and fixed to the container wall using tape. The boxes were tightly closed by lids that contained a lockable opening for water supply. Pieces of cellulose paper were added as a potential food source. The boxes were maintained outdoors at ambient temperatures (cf. enclosures; Chapter 3.1.6.2). 32 individuals each (three females, three males, eight subadults and 18 immatures) were collected on the banana plantation and in the inundated forest of Marchantaria Island in April 2003 and placed into one of the boxes. Animals were left to get acclimatised to their new environments and to reproduce for one month. Observations during this time served as control and since the treatment

only started in the second month. For gradual inundation, 25 ml of water per week were sprayed over the microterrain in each box, simulating rain. The ground became flooded from the third month onwards. After the fourth month, irrigation was stopped and the water level left to fall via evaporation, which took another five months. During both control and treatment, the migration behaviour was monitored by counting adult and juvenile specimens visible on litter, bark and container walls at weekly intervals for a period of five months. The number of individuals remaining in the soil, however, could not be examined.

3.1.6.2 Reproduction Experiment

To identify the factors regulating reproduction, i.e. inducing a univoltine life cycle in *P. obliterata* during the aquatic phase, animals were cultivated under different controlled conditions in enclosures (see below). From April to October 2003, experimental rearing and biological observations were made using individuals sampled from the inundation forest population of Marchantaria Island and the upland population on the plantation at CPAA/Embrapa, since *P. obliterata* were most abundant at these two sites. This way, exemplary comparative analyses between individuals from seasonally flooded and permanent non-flooded biotopes were possible.

Four external factors were considered for a potential impact on reproduction. Firstly, habitat substrate (soil/litter versus bark), since the presence of soil is regarded as a primary factor inducing oviposition in *Pentacomia egregia*, a resident cicindelid beetle also showing a univoltine life cycle in floodplains (Amorim et al. 1997). Secondly, the mean air temperature (variation of day and night temperature decreases due to the buffering effect of the water body), which can act as a synchroniser for gonad dormancy and delayed maturation, as observed in females of *P. egregia* (Amorim et al. 1997). The third factor of concern is the relative humidity, given that millipedes are very sensitive in terms of moisture (Hopkin & Read 1992), particularly juveniles due to their thinner exoskeleton. In the resident millipede *Pycnotropis tida*, the univoltine life cycle during flooding is caused by the mortality of juveniles, mainly as a result of the dry conditions while exposed on tree trunks (Vohland & Adis 1999). The fourth factor considered is nutrition, since both food resources and the availability of minerals are possibly restricted on bark compared to soil and litter. Food deprivation can affect the reproductive investment and result in lower fecundity and decreased reproductive

success in arthropods (Stadler 1995; Momen 1994; Rankin et al. 1997). The quality of food was shown to affect female fertility in the millipede *Polydesmus angustus* (David & Celerier 1997). Calcium is of particular importance during postembryonic development for the formation of an exoskeleton in immature millipedes subsequent to ecdysis (Hopkin & Read 1992; Chapter 1.2.1.5). However, at least some of the local polydesmidan millipedes are capable of accumulating sufficient amounts of calcium from dead wood (Vohland et al. 2003).

The effects of the different factors on mating, the reproductive output, postembryonic development of offspring and the mortality rate of adults were investigated in a nested design to test for possible interactions among treatment, habitat substrate and population. Ten replicates, i.e. pairs of *P. obliterata*, per population were used in each of the four different treatments (Table 5). The respective pairs were kept separately in square (6x5x3 cm) or round (5.5 cm in diameter, 1.5 cm in height) plastic boxes closed by lids. In the control and all other treatments except the 'no plaster' one, the bottom was coated (approx. 0.5 cm in height) with a moist mixture of plaster (nine parts plaster and one part charcoal powder) to provide for an adequate, constantly high humidity. In addition, the plaster layer ensures a sufficient calcium supply. In the 'no plaster' treatment, the bottom was covered instead with moist filter paper. The specimens were cultivated on moist earth substrate (eight parts vegetable mould, one part bark mulch and one part ground leaves of chestnut: *Castanea sativa* Mill.; Fagaceae) and bark pieces (approx. 3.5x3.5 cm; obtained from native trees colonised by *P. obliterata* during inundation: *Macrolobium acaciifolium* Benth., Fabaceae, and *Eschweilera ovalifolia* Nied., Lecythidaceae), respectively, to simulate the habitat substrate during the terrestrial and aquatic phase. The plaster layer, filter paper and earth substrate were remoistened with water droplets every two to four days to maintain a water saturated atmosphere. Pursuing the same time schedule, the millipedes in all treatments except for the 'no food' one were fed three times a week with moistened flakes of the protein-rich fish food 'Red Tetramin' (min. crude protein: 45 %, min. crude fat: 5 %, max. crude fibers: 2 %, max. moisture: 6 %; 'Tetra Werke', Melle, Germany), which was offered on pieces of cellulose paper (approx.1x1 cm) to facilitate the replacement of old food. All these basic rearing conditions prove to be adequate for the cultivation of *P. obliterata* (Adis et al. 2001). The animals in all treatments except for the 'constant temperature' one were maintained in 'enclosures', i.e. outdoors at ambient temperature (cf. Chapter 1.1.2), sheltered against solar radiation and rainfall by a roof (Fig. 15a). To

protect the millipedes from predatory ants, all boxes were arranged on supports within plastic basins filled with water (Fig. 15b). The animals in the 'constant temperature' treatment were kept in a climate chamber at a constant temperature of 24 °C, like in the investigations on gonad dormancy in *P. egregia* (see above).

Table 5. Experimental design of the rearing experiments with *P. obliterata*. Treatments were performed separately for animals from a floodplain (Marchantaria Island) and an upland (CPPA/Embrapa) population. For each population, ten replicates of one male and female were used per treatment. Abbreviations: e, earth; b, bark; and T, temperature.

Treatment	Habitat substrate	Temperature	Plaster	Nutrition
control e	earth	ambient T	with plaster	with food
control b	bark	ambient T	with plaster	with food
constant T e	earth	24 °C	with plaster	with food
constant T b	bark	24 °C	with plaster	with food
no plaster e	earth	ambient T	no plaster	with food
no plaster b	bark	ambient T	no plaster	with food
no food e	earth	ambient T	with plaster	no food
no food b	bark	ambient T	with plaster	no food

Figure 15a-b.: Cultivation of *Poratia obliterata*. **a** – the animals were kept in plastic boxes and maintained in outdoor enclosures at ambient temperature, sheltered by a roof. **b** – the boxes were arranged on supports within plastic basins filled with water (Pictures: N.G.R. Bergholz).

Before being exposed to the respective reproduction experiments, female and male subadults as well as immature individuals representing the 6th developmental stage of both sexes were reared under the conditions of both 'constant temperature' and control treatment until they reached adulthood. Animals dying beforehand, i.e. during development and ecdysis, were replaced, with a restriction to the immediate availability of specimens of the respective age and sex from field collections. Collected adults from the field were only used in the 'no plaster' and 'no food' treatments.

The observation period of the reproductive experiment started with adult pairs and lasted six months during which the occurrence of egg chambers, offspring and subsequent developmental stages were monitored in the four different treatments. Using a stereo microscope with low illumination (Chapter 3.1.5), the boxes were checked weekly, with dead animals being removed. Millipedes were mainly located in the free area around the offered food and on the surface of bark and earth substrate, the latter hardly being searched for animals to prevent moulting individuals from damage.

If adults died too rapidly to potentially reproduce, the respective individuals were replaced. In each of these events, the calculation of time was restarted when a complete mature pair was present. Due to the delay caused by lack of availability from collections in the field, not all replicates could be monitored over a period of six months. Similarly, due to technical problems with the climate chamber, the 'constant temperate' treatment had to be restarted and thus did not comprise six but only three months of observation (and up to four months for single replicates).

3.1.7 Data Analysis and Statistics

Unless mentioned otherwise, descriptive statistics and analyses were performed using STATISTICA, version 6 (StatSoft Inc. 2001).

Given that millipedes on tree trunks are distributed contagiously ('over dispersed'), the median is superior to the mean to express central tendency in the positively skewed distributions (Zar 1996; Sachs 1999). To estimate variation in both group size and position of aggregated *P. obliterata* among sampled trees and populations, Kruskall-Wallis tests were used to study median and variance homogeneity and to identify significant differences. Kolmogorov-Smirnov tests were applied to compare the distribution of group size, position and the occurrence of other species in aggregated *P. obliterata* in 2002 and 2003. The course and duration of the aquatic phase, i.e. the

residence time the millipedes spent on tree trunks, was calculated considering the minimum flood periods of individual sample trees (Chapter 3.1.3). The relation between gregarious behaviour and relative position of millipedes as well as correlations of size, position and other species' share in groups of *P. obliterata* with residence time and regional precipitation per month (meteorological data obtained from INMET, Manaus) were evaluated in rank correlation analyses of Kendall's tau (Dytham 2003). To eliminate any significant interactions between variables on a particular relationship, partial correlations were used as necessary (partial correlation coefficients express the correlation between two variables, assuming that other variables are hold at constant values). Using Microsoft EXCEL, partial correlation coefficients were calculated from simple Kendall's correlation coefficients according to Sachs (1999, p. 571) and Zar (1996, p. 421). Since Kolmogorov-Smirnov tests revealed significant differences between the years, all correlations were performed separately for the datasets of 2002 and 2003. To evaluate the effects of outliers, supplementary correlations were computed excluding these data.

Kendall's tau correlations were used to estimate a significant increase or decrease in the monthly numbers of different developmental stages of *P. obliterata* monitored in the field during the aquatic phase. Correlations were estimated for the datasets of 2002, 2003 and both years combined. Tests were performed for single populations as well as for pooled data from the three inundation forest populations. For the upland population, additional Kendall correlations with local climatic factors (precipitation and insolation) were performed.

To estimate differences in the occurrence of male and female *P. obliterata* during field observations, Mann-Whitney tests were performed for the datasets of 2002 and 2003. Chi-square goodness of fit was used to test for sex ratio differences among years and populations. Kruskal-Wallis tests were performed to test for differences in sex ratio in the course of the aquatic phase.

Differences in the proportion of sampled females containing mature, immature as well as no eggs in August and September 2003 were evaluated by a chi-square goodness of fit. Due to the number of observations, this analysis was restricted to females of the inundation forests. To test for monthly deviations in the numbers of mature and immature eggs per female, Kolmogorov-Smirnov tests were applied for all populations. Kruskal-Wallis tests were used to estimate differences in these quantities among populations and biotope types.

The percentage of water content in different habitat substrates (decaying banana stems; bark pieces collected at various heights from the sunny and shady sides of trees) was calculated by means of the fresh to dry matter ratio. Using EXCEL, these proportional data were normalised by an arcsine transformation, converting relative frequencies into angles (Sachs 1999). Statistical analyses were performed selecting habitat substrate as categorical factor and water content as dependent variable. To test for significant differences in humidity, a one-way analysis of variance (ANOVA) with Tukey post-hoc comparisons was conducted, since Tukey's method maintains the error rate of the pre-established α level (Brown 2005). Residuals were shown to be normally distributed.

The total number of millipedes in the flooding experiment cannot be referred to since data for the specimens dwelling in the soil is not available. Instead of relative frequencies, the maximum number of adults and juveniles observed at a specific location (litter, bark and container walls) per month was considered for interpretation. The proportion of the respective abundance in the first month (control) to that in the second month (treatment) was used to compare relative migratory responses to flooding. In the rare event of zero values, these had to be transformed into numerical values of 1 for these estimations. To test for differences among two proportions within and between populations, a procedure analogous to the chi-square test was employed (Zar 1996), with values being calculated in EXCEL (Example 23.23, page 554 in Zar 1996) and significances verified using $Z_{0.05(2)} = 1.9600$. A conventional chi-square analysis to test for differences in the number of individuals on litter and bark/container wall in the course of the whole observation period (month 0 to 5) was performed.

Using the software R, version 2.3.0 (Ihaka & Gentleman 1996; Venables et al. 2006) a linear mixed effect model was employed to test for significant effects within the nested design of the reproduction experiment. The occurring developmental stages per month were chosen as a dependent variable. The grouping variables treatment (control, no plaster, constant temperature and no food), habitat substrate (earth and bark) and their interaction nested within population (Marchantaria Island and CPPA/Embrapa) were defined as fixed effects; the month and replicate pair, both nested within population, represented random effects. A multinominal logistic regression was performed with the software SPSS, version 11.5 (SPSS Inc. 2002) to estimate the particular influence of treatment, habitat substrate and population on the mortality of both the maturing individuals reared in advance of, and the adults reared in, the reproduction experiment. Control (treatment), earth (habitat substrate) and EB (population) were applied as a

reference group to estimate logistic regression coefficients (effect coefficients) and odds ratios.

For all statistical tests a significance level of 5 % was accepted.

Except for figures concerning the phenology of *P. obliterata*, which were constructed using EXCEL, all graphs were constructed using SigmaPlot, version 8.

3.2 Results

3.2.1 Abiotic Factors

3.2.1.1 Precipitation

The last months in 2002 and the first months in 2003 were characterised by a moderate El Niño (Latif, pers. commun.).

On the upland banana plantation at CPPA/Embrapa, annual precipitation as measured by the local weather station was notably lower in 2003 (1,958 mm) than in 2002 (2,937 mm). The average monthly distribution of rainfall also differed between years (Fig. 16a). Compared to 2002, the mean precipitation in 2003 was remarkably low from January to March and also minor in July and October to December. Only in September did monthly rainfall in 2003 exceed the values in 2002.

The annual local precipitation adjacent to whitewater inundation forests as quantified by INMET (National Meteorological Institute at Manaus; weather station at CEASA harbour next to the Negro River; 03°07'S 059°57'W) was only slightly lower in 2003 (2,288 mm) than in 2002 (2,410 mm). This shows that rainfall was lower in whitewater inundation forests than on Terra firme in the climatically normal year 2002 (cf. Ribeiro & Adis 1984), but higher in 2003. The distribution of the mean monthly precipitation differed in the two years (Fig. 16b). In 2003, rainfall was lower than in 2002 for January, March, May to June, September to October and December, but higher for February, April and July to August. Monthly precipitation was more evenly distributed in 2003 than in 2002, lacking both the rainfall peak in May to June and a rapid decline thereafter in July to August.

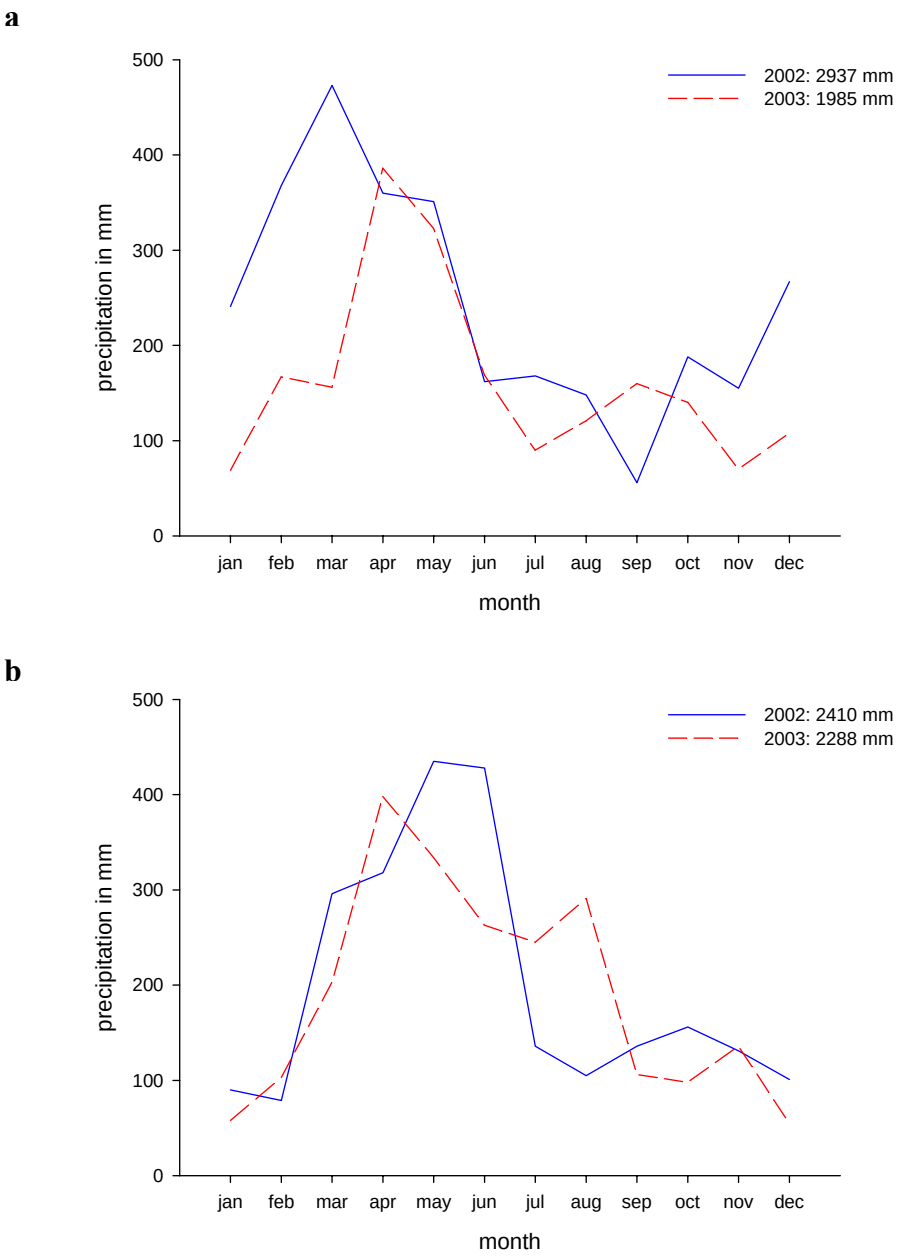

Figure 16a-b. Average monthly precipitation recorded at weather stations near Manaus in 2002 and 2003. **a.** upland region (CPPA/Embrapa). **b.** adjacent to inundation forests (INMET: CEASA at Negro River).

3.2.1.2 Water Level

The Negro River volume was less in 2003 than in 2002 (Fig. 17). As a result, both water level rise and inundation of adjacent areas in 2003 were delayed for approximately one month compared to 2002. Accordingly, the deviation between the maximum and minimum water levels was also lower in 2003 (9.3 m) than in 2002 (11.7 m).

Given that rise and fall of the water level of the Solimões River are comparable to those of the Negro River (Adis, pers. commun.), field data obtained at Marchantaria Island

(Solimões River) as well as Lake Janauarí (Solimões River & Negro River) can be correlated with water level fluctuations of the Negro River.

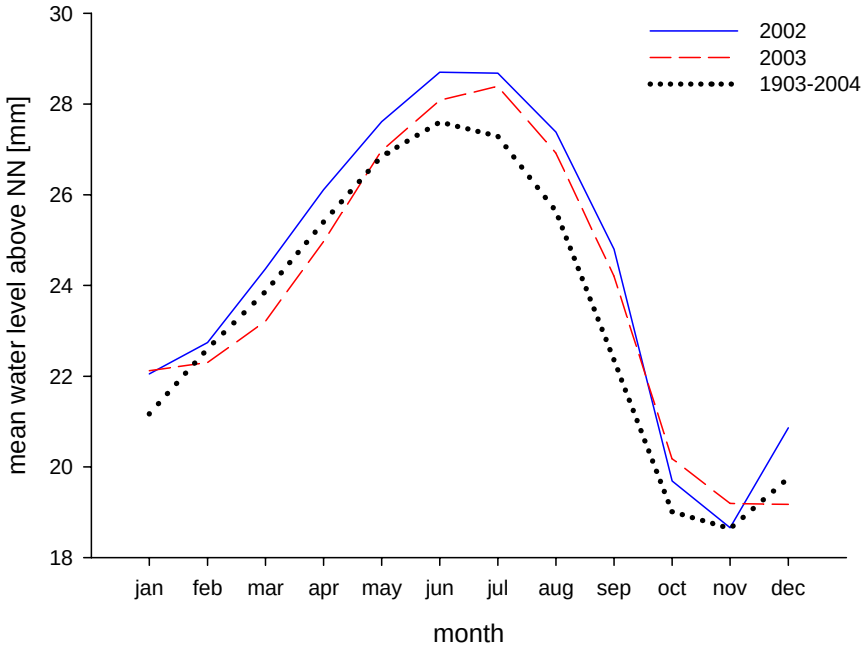

Figure 17. Average water levels of the Negro River in 2002, 2003 and for 1903 to 2004 as measured at the harbour of Manaus.

3.2.1.3 Minimum Flood Period at Study Sites

Since the study sites in the three inundation forests were situated at different altitudes, the duration of the respective flood period varied as well. The longest submersion phase was observed for the study site on Marchantaria Island (Várzea). Due to the delayed and less pronounced rise of the water level in 2003, the study sites were inundated for an average of 25 to 35 days less in 2003 than in 2002 (Fig. 18).

The sampling area on Marchantaria Island was flooded from March to September for at least 200 days in 2002 and 173 days in 2003, i.e. from a water level of 23.92 m above NN onwards. Two sample trees were inundated for ≥ 218 days in 2002 (22.02.-28.09.02, water level threshold: 23.25 m) and for ≥ 190 days in 2003 (18.03.-23.09.03); four trees for ≥ 202 days in 2002 (07.03.-24.09.02, water level threshold: 23.85 m) and for ≥ 174 days in 2003 (30.03.-19.09.03); seven trees for ≥ 198 days in 2002 (10.03.-23.09.02, water level threshold: 24.00 m) and for ≥ 171 days in 2003 (01.04.-18.09.03); and one tree was flooded for ≥ 174 days in 2002 (26.03.-15.09.02, water level threshold: 24.95 m) and for ≥ 150 days in 2003 (15.04.-11.09.03) (Table 1).

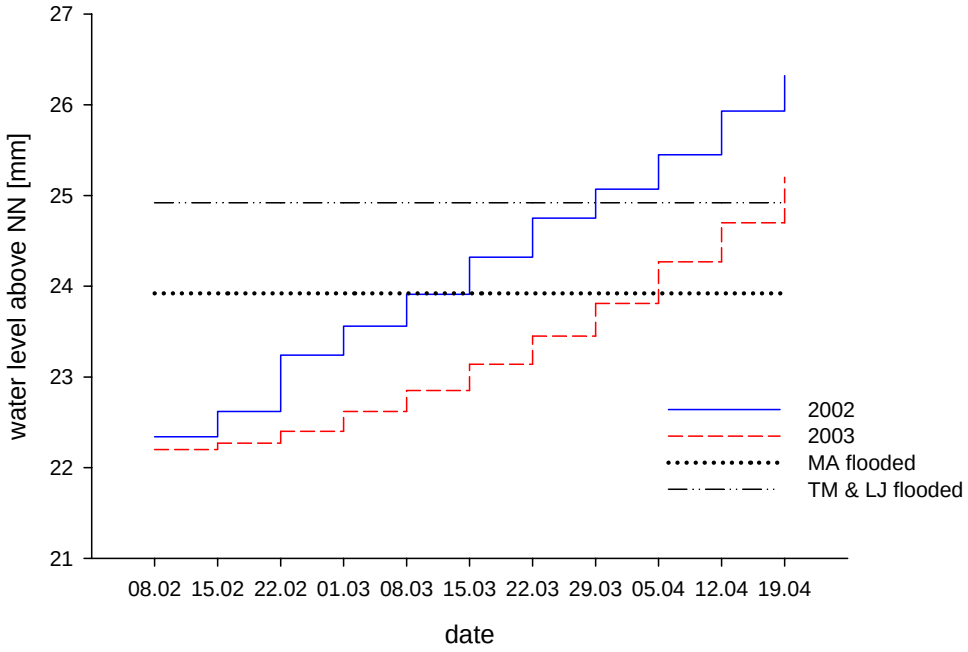

Figure 18. Weekly rise in water level of the Negro River in early 2002 and 2003 (measured at the harbour of Manaus). The average water level thresholds at which sampling areas in the different inundation forests are flooded are indicated by horizontal lines. Abbreviations: MA, Marchantaria Island; TM, Tarumã Mirím River; and LJ, Lake Janauarí.

At Tarumã Mirím River (Igapó), the minimum inundation period for the study sites averaged 171 days in 2002 (March to September) and 147 days in 2003 (April to September), the water level threshold being 24.92 m. Four of the sampled trees were flooded for ≥ 195 days in 2002 (12.03.-22.09.02, water level threshold: 24.13 m) and for ≥ 169 days in 2003 (03.04.-18.09.03); four others for ≥ 183 days in 2002 (21.03.-19.09.02, water level threshold: 24.65 m) and for ≥ 157 days in 2003 (11.04.-14.09.03); one for ≥ 171 days in 2002 (28.03.-14.09.02, water level threshold: 25.05 m) and for ≥ 149 days in 2003 (16.04.-11.09.03); another for ≥ 142 days in 2002 (15.04.-03.09.02, water level threshold: 26.11 m) and ≥ 119 days in 2003 (03.05.-29.08.03); and three were inundated for ≥ 134 days in 2002 (20.04.-31.08.02, water level threshold: 26.38 m) and for ≥ 112 days in 2003 (07.05.-26.08.03) (Table 2).

Similar to the Igapó site, the flood period in the sampling area at Lake Janauarí (Várzea & Igapó) averaged 173 days in 2002 (March to September) and 148 days in 2003 (April to September) at the minimum, the critical water threshold being 24.94 m. Two sample trees were inundated for ≥ 218 days in 2002 (22.02.-28.09.02, water level threshold: 23.25 m) and for ≥ 190 days in 2003 (18.03.-23.09.03); two others for ≥ 198 days in 2002 (10.03.-23.09.02, water level threshold: 24.00 m) and for ≥ 171 days in 2003

(01.04.-18.09.03); four trees for ≥ 188 days in 2002 (17.03.-20.09.02, water level threshold: 24.43 m) and for ≥ 163 days in 2003 (07.04.-16.09.03); two trees for ≥ 174 days in 2002 (26.03.-15.09.02, water level threshold: 24.95 m) and for ≥ 150 days in 2003 (15.04.-11.09.03); one tree for ≥ 157 days in 2002 (06.04.-09.09.02, water level threshold: 25.56 m) and for ≥ 135 days in 2003 (24.04.-05.09.03); two trees for ≥ 144 days in 2002 (14.04.-04.09.02, water level threshold: 26.00 m) and for ≥ 122 days in 2003 (02.05.-31.08.03); two others for ≥ 135 days in 2002 (19.04.-31.08.02, water level threshold: 26.36 m) and for ≥ 112 days in 2003 (07.05.-26.08.03); and one tree was flooded for ≥ 119 days in 2002 (28.04.-24.08.02, water level threshold: 26.90 m) and for ≥ 96 days in 2003 (16.05.-18.08.03) (Table 3).

3.2.1.4 Humidity in the Microhabitat

Microhabitats on the upland plantation and in the inundation forests of Marchantaria Island differ markedly in their relative humidity, as defined by the water content of the habitat substrate (Fig. 19). Whereas the water content of decaying plant material on the plantation averaged 87 %, the moisture level was significantly lower in bark microhabitats on tree trunks in the Várzea (Table 6). The average water content of collected bark pieces ranged from 23 to 36 %, decreasing with height above the water line but not with insolation (Fig. 19). Bark microhabitats close to the water line, i.e. up to a distance of 10 cm, were most humid. Moisture declined significantly with increasing height, but did not differ significantly at distances of 20 and 50 cm (Table 6). Insolation apparently had no significant desiccative effect, since the water content of bark pieces collected from sunny sites of trees was not significantly lower.

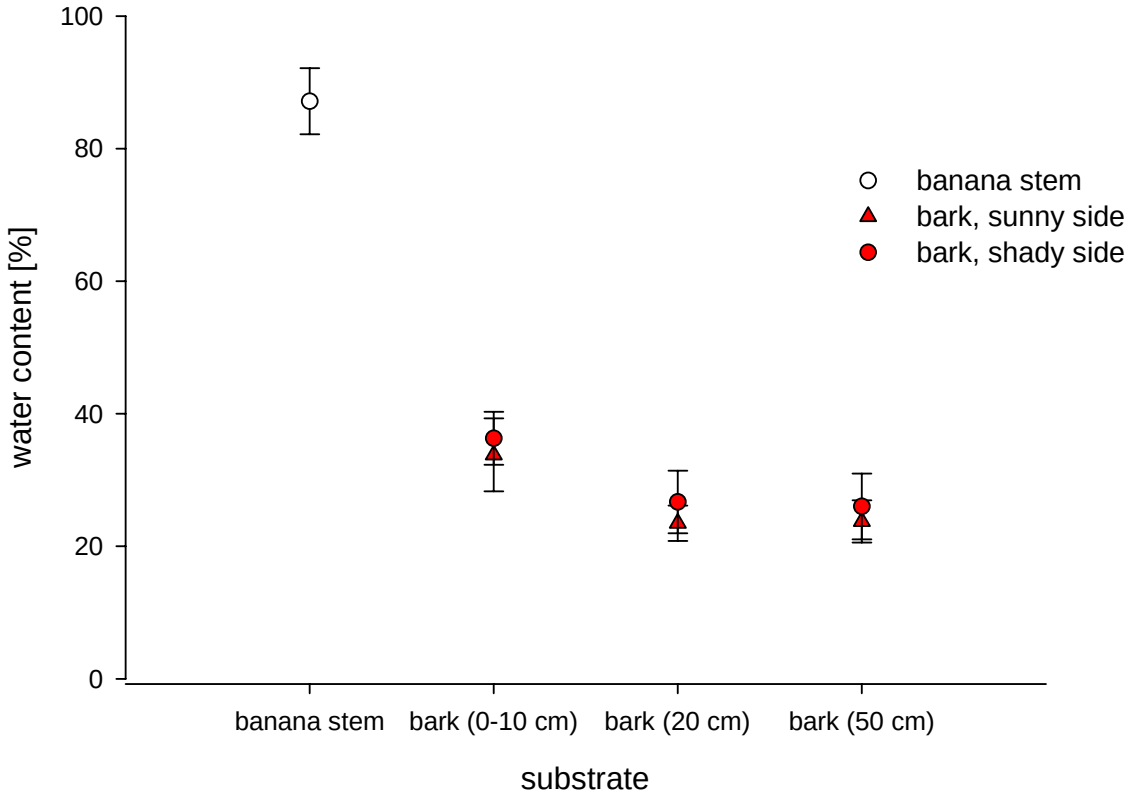

Figure 19. Humidity in microhabitats (as percentage of water content in the habitat substrate) on the plantation at CPPA/Embrapa (decaying banana stems) and on tree trunks within the inundation forest of Marchantaria Island (bark pieces at various heights above the water line: 0 to 10, 20 and 50 cm, from sunny and shady sides of trees). (n = 30; bars indicate standard deviation, levels of significant differences are given in Table 6).

Table 6. Results of a one-way analysis of variance (ANOVA), Tukey post hoc tests: multiple comparisons of the water content of seven habitat substrates (decaying banana stems; bark pieces at different heights above the water line from sunny and shady sides of trees). Bold numbers indicate significant differences accepting a 5 % significance level.

Substrates		***P*- value**
banana stem	bark, 0-10 cm height, shady side	**0,000**
banana stem	bark, 0-10 cm height, sunny side	**0,000**
banana stem	bark, 20 cm height, shady side	**0,000**
banana stem	bark, 20 cm height, sunny side	**0,000**
banana stem	bark, 50 cm height, shady side	**0,000**
banana stem	bark, 50 cm height, sunny side	**0,000**
bark, 0-10 cm height, shady side	bark, 0-10 cm height, sunny side	0,400
bark, 0-10 cm height, shady side	bark, 20 cm height, shady side	**0,000**

Substrates		*P*- value
bark, 0-10 cm height, shady side	bark, 20 cm height, sunny side	**0,000**
bark, 0-10 cm height, shady side	bark, 50 cm height, shady side	**0,000**
bark, 0-10 cm height, shady side	bark, 50 cm height, sunny side	**0,000**
bark, 0-10 cm height, sunny side	bark, 20 cm height, shady side	**0,000**
bark, 0-10 cm height, sunny side	bark, 20 cm height, sunny side	**0,000**
bark, 0-10 cm height, sunny side	bark, 50 cm height, shady side	**0,000**
bark, 0-10 cm height, sunny side	bark, 50 cm height, sunny side	**0,000**
bark, 20 cm height, shady side	bark, 20 cm height, sunny side	0,099
bark, 20 cm height, shady side	bark, 50 cm height, shady side	0,997
bark, 20 cm height, shady side	bark, 50 cm height, sunny side	0,164
bark, 20 cm height, sunny side	bark, 50 cm height, shady side	0,340
bark, 20 cm height, sunny side	bark, 50 cm height, sunny side	1,000
bark, 50 cm height, shady side	bark, 50 cm height, sunny side	0,474

3.2.2 Morphology

3.2.2.1 *Poratia obliterata*

Specimens of *P. obliterata* from the sample sites (Fig. 50) represented bisexual populations and were identical in all morphological characters, e.g. body size (5 to 7 mm), number of body segments (20), lateral lobulations on paraterga 16 to 19 and the structure of the gonopod telopodite (cf. Golovatch & Sierwald 2001).

3.2.2.2 Other Millipede Species

Four small millipede species co-occurring with *P. obliterata* were identified based on morphology:

Poratia insularis Kraus 1960 (Diplopoda: Pyrgodesmida: Pyrgodesmidae; body size: 5 to 7 mm), a congener of *P. obliterata* previously known only from the type locality, i.e. Muyuy Island in the Solimões River near Iquitos, Peru (Kraus 1960). So it has been recorded in Central Amazonia, and in Brazil as a whole, for the first time (Bergholz et al. 2005).

Cutervodesmus adisi Golovatch 1992 (Diplopoda: Pyrgodesmida: Fuhrmannodesmidae; body size: 4.5 to 6 mm), so far only known from the blackwater inundation forest at Tarumã Mirím River (Golovatch 1992).
Docodesmus amazonicus Golovatch 1997 (Diplopoda: Pyrgodesmida: Pyrgodesmidae; body size ca. 8.5 mm), so far only known from Terra firme, i.e. secondary forest at Tarumã Mirím River (Golovatch 1997a).
Myrmecodesmus hastatus Schubart 1945 (Diplopoda: Pyrgodesmida: Pyrgodesmidae; body size 5 to 8 mm), a species widely distributed in Brazil (Pará, Rio de Janeiro, Distrito Federal) and Argentina, probably as a result of association not only with human settlements, but also ant nests and termites (Golovatch 1996). It has been recorded in Central Amazonia for the first time (Bergholz et al. 2004).

3.2.3 Ethology

3.2.3.1 Inundation Forests

3.2.3.1.1 Seasonal Vertical Migration

P. obliterata specimens in the inundation forests dwelled on the ground, i.e. within litter, soil and occasionally dead wood, during the terrestrial phase and avoided drowning during the aquatic phase by seasonal vertical migrations. The animals did not climb tree trunks beforehand, but only escaped when directly forced by the rising waters. In contrast to other resident species (Adis 1981; Adis), all developmental stages of *P. obliterata* showed trunk ascent, but advanced stages (juveniles of the 6th stage and subadults) definitely outnumbered the others and thus can be termed migratory stages. At the end of the inundation period, the animals returned to the ground and ceased to reside on trees.
P. obliterata generally favoured trees with rather coarse and loose-fitting bark for trunk ascents, since individuals took refuge under bark, presumably to protect themselves from sun and predatory arthropods. The animals preferred small to medium-sized bark pieces for shelter, probably because larger pieces often harboured large predators such as chilopods (Chilopoda) and spiders (Arachnida). The sampled trees were also colonised by seasonally migrating ants (Formicidae). Given that individuals of *P. obliterata* were occasionally found together with some small ants, however, there seemed to be no interference at moderate ant densities. In contrast, on trees with permanent termite (Isoptera) colonies, *P. obliterata* were only rarely encountered.

Due to their inconspicuous coloration and small size, the millipedes were generally well disguised. When being sampled, individuals tended to adhere to the bark or rapidly escape into small crevices, probably the same reaction they would show in the presence of predators.

On Marchantaria Island, specimens were collected on 16 trees comprising four different tree species, namely *Macrolobium acaciifolium* [Benth.] Benth., Fabaceae (Várzea, Igapó and mixedwater); *Eschweilera ovalifolia* [DC.] Nied., Lecythidaceae (Várzea and mixedwater); *Vitex cymosa* Bertero ex Spreng., Verbenaceae (Várzea and mixedwater) and *Nectandra amazonum* Nees, Lauraceae (Várzea, Igapó and mixedwater) (Table 1).

Most likely due to its loose bark, the majority of millipedes were encountered on *M. acaciifolium*. As a result of relatively dense bark, some millipedes on *E. ovalifolia* were located outside, hiding in moist crevices. The bast-like bark of *V. cymosa* readily absorbs water, usually being more humid and overgrown by fungi.

P. obliterata were occasionally found associated with its congener *P. insularis* (Bergholz et al. 2005), but only on a single tree trunk (n° 15) located on the lakeside opposite to the others.

At other Várzea sites (Table 17), *P. obliterata* were also collected on *Mabea nitida* Spruce ex Benth., Euphorbiaceae (Careiro Island and Lake Janauacá;Várzea, Igapó and mixedwater) and *Cassia leiandra* Benth., Fabaceae (Lake Janauacá; Várzea, Igapó) as well as few other trees which could not be specifically identified. The millipedes were in part also encountered on dead but standing wood, i.e. upright decaying tree trunks. *P. obliterata* co-occurred with *P. insularis* (Careiro Island and Lake Janauacá) as well as other small millipede species, namely *Cutervodesmus* sp. (Fuhrmannodesmidae) on Careiro Island, Paciência Island and at Lake Janauacá and *Docodesmus* sp. (Pyrgodesmidae) on Careiro Island and at Lake Janauacá.

At Tarumã Mirím River, *P. obliterata* were sampled on ten trees representing four different species: *M. acaciifolium, Tabebuia barbata* [E. Mey] Sandw., Bignoniaceae (Várzea, Igapó and mixedwater), *Aldina latifolia* Spruce ex Benth., Fabaceae (Igapó and mixedwater), and *Calophyllum brasiliense* Camb., Clusiaceae (Várzea, Igapó and mixedwater); as well as on four trees which could not be specifically identified (Table 2). One of the sampled *A. latifolia* specimens was a dead tree trunk (n° 14).

Most individuals were again found on *M. acaciifolium*.

P. obliterata were found associated with *Cutervodesmus adisi* (Golovatch 1992) on six of the 14 trees examined (n° 3 to 4, 8 to 9, 11 and 16) and also co-occurred with *Docodesmus amazonicus* (Golovatch 1997a) on a single tree (n° 5).

At other Igapó locations (Table 17), *P. obliterata* were also found on *C. leiandra* (Puraquequara River), *M. nitida* (Anavilhanas Islands) and *Acosmium nitens* (Vogel) Yakovlev, Caesalpiniaceae (Anavilhanas Islands; Várzea, Igapó and mixedwater), as well as on several trees which could not be specifically identified, partly representing standing dead wood. *P. obliterata* were found associated with *P. insularis* on the Anavilhanas Islands and co-occurred with *Cutervodesmus* sp. and *Docodesmus* sp. at the Puraquequara River.

At Lake Janauarí, specimens were collected on 17 trees representing four different tree species, *M. acaciifolium*, *E. ovalifolia*, *C. leiandra*, *C. brasiliense*, as well as on four trees which could not be specifically identified, partly as standing dead wood (Table 3). Again, most of the specimens were encountered on *M. acaciifolium*.

P. obliterata were found associated with *P. insularis* on nine of the 17 trees examined (n° 2 to 3, 7, 11 to 12, 14 to 16 and 18) and co-occurred with *C. adisi* on three of the trees (n° 5, 15 to 16) and/or with *D. amazonicus* on ten of the trees (n° 2, 5, 7 to 9, 11, 15 to 18).

3.2.3.1.2 Preferred Microhabitats and Gregarious Behaviour

During the aquatic phase, *P. obliterata* specimens in the different inundation forests dwelled in aggregations, which also comprised individuals of other small millipede species, close to the water line (Figs 20-22). Most of the groups, including the largest, were observed near the water line at distances of approximately 10 to 20 cm, while only sporadic, mainly single individuals were found at further distances and up to 100 to 150 cm of height. Only one single male inhabited the moist substrate of an epiphyte at a height of 200 cm (Fig. 21).

Kruskal-Wallis tests revealed significant median and variance inhomogeneity ($P < 0.050$) of both size and position in groups of *P. obliterata* among the sampled trees on Marchantaria Island (MA) and at Tarumã Mirím River (TM). At Lake Janauarí (LJ), significant median homogeneity but variance inhomogeneity of overall group size, as well as significant median and variance inhomogeneity of the relative position of groups, among trees were observed ($P < 0.050$). Multiple comparisons showed that

aggregation sizes on MA were significantly different among tree 7 and trees 11 and 12 ($P < 0.050$; Fig. 23a). The relative position of groups was significantly distinct between tree 14 and all other trees (except for n° 5, 6, 11, 15 and 16); between tree 15 and trees 1 to 4; and between tree 1 and tree 11 ($P < 0.050$; Fig. 23b). At TM, aggregation sizes varied significantly among tree 12 and trees 2 and 3, and among tree 2 and tree 4 ($P < 0.050$; Fig. 24a). The height above the water line was significantly distinct only between tree 12 and trees 4 and 6 ($P < 0.050$; Fig. 24b). Aggregation sizes at LJ were significant different among tree 7 and 11 ($P < 0.050$, Fig. 25a). The relative position varied significantly between tree 3 and several trees (n° 5, 8, 10 and 15); between tree 8 and trees 17 and 18; as well as between tree 10 and other trees (n° 7, 11, 17 and 18) ($P < 0.050$, Fig. 25b).

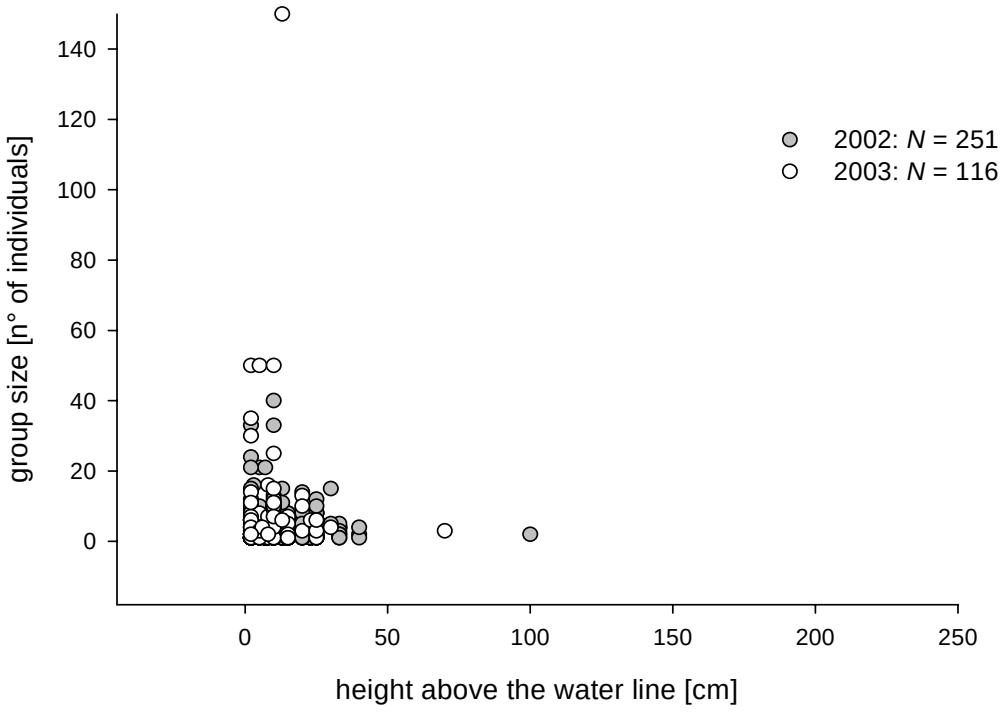

Figure 20. Dispersion of aggregated *P. obliterata* on tree trunks on Marchantaria Island during the aquatic phases in 2002 and 2003: group size (rarely associated with *P. insularis*, once with *C. adisi*) and position relative to the water line. Number of groups in different heights (in cm) as indicated by indices (N_2-N_{100}): $N_2 = 101$; $N_3 = 1$; $N_5 = 25$; $N_6 = 1$; $N_7 = 21$; $N_8 = 8$; $N_{10} = 60$; $N_{13} = 29$; $N_{15} = 39$; $N_{18} = 2$; $N_{20} = 16$; $N_{23} = 21$; $N_{25} = 26$; $N_{30} = 3$; $N_{33} = 9$; $N_{40} = 3$; $N_{70} = 1$; $N_{100} = 1$.

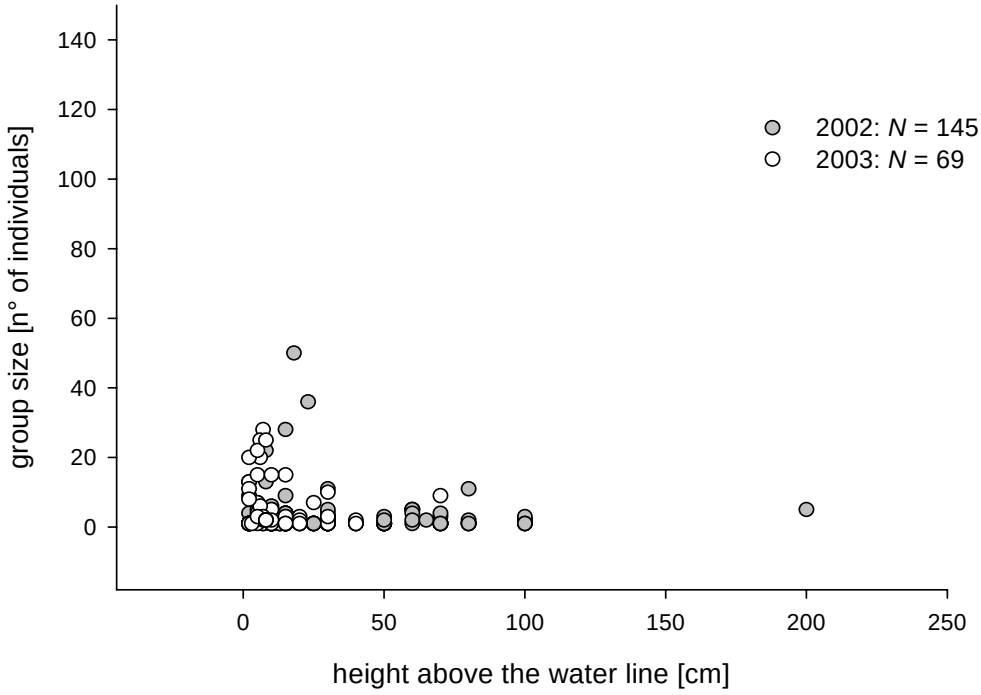

Figure 21. Dispersion of aggregated *P. obliterata* on tree trunks at Tarumã Mirím River during the aquatic phases in 2002 and 2003: group size (partly associated with *C. adisi*, rarely with *D. amazonicus*) and position relative to the water line. Number of groups in different heights (in cm) as indicated by indices (N_2-N_{200}): N_2 = 18; N_3 = 1; N_5 = 9; N_6 = 3; N_7 = 6; N_8 = 8; N_{10} = 30; N_{13} = 6; N_{15} = 36; N_{18} = 2; N_{20} = 9; N_{23} = 1; N_{25} = 8; N_{30} = 27; N_{40} = 6; N_{50} = 13; N_{60} = 6; N_{65} = 1; N_{70} = 9; N_{80} = 6; N_{100} = 8; N_{200} = 1.

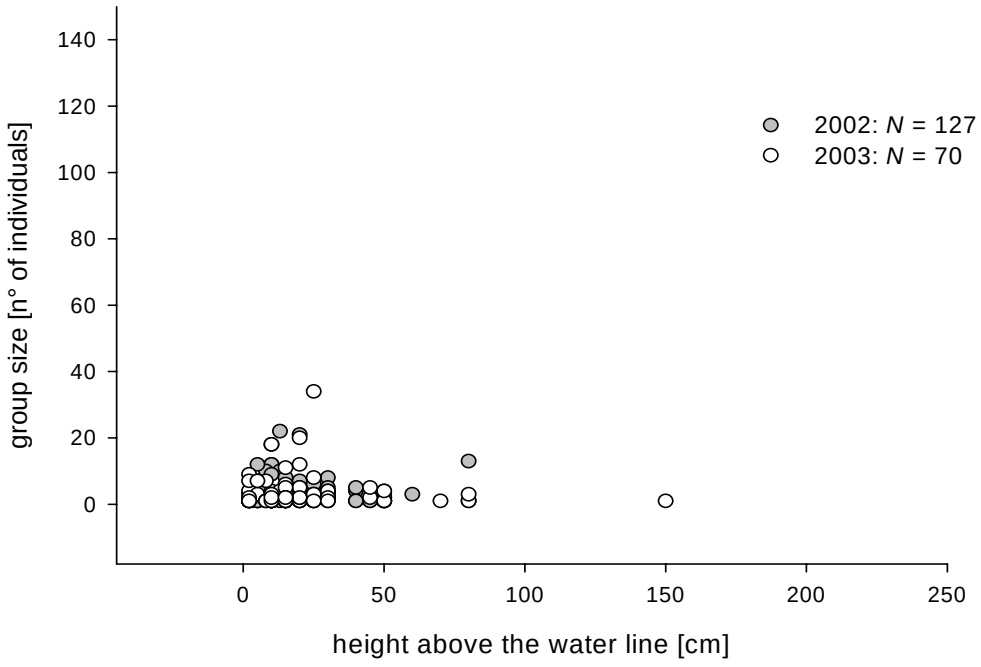

Figure 22. Dispersion of aggregated *P. obliterata* on tree trunks at Lake Janauarí during the aquatic phases in 2002 and 2003: group size (partly associated with *P. insularis*, *C. adisi* and *D. amazonicus*) and position relative to the water line. Number of groups in different heights (in cm) as indicated by indices (N_2-N_{150}): N_2 = 16; N_3 = 1; N_5 = 17; N_7 = 2; N_8 = 5; N_{10} = 34; N_{13} = 5; N_{15} = 31; N_{20} = 23; N_{23} = 4; N_{25} = 15; N_{30} = 15; N_{40} = 4; N_{45} = 4; N_{50} = 14; N_{60} = 1; N_{70} = 1; N_{80} = 4; N_{150} = 1.

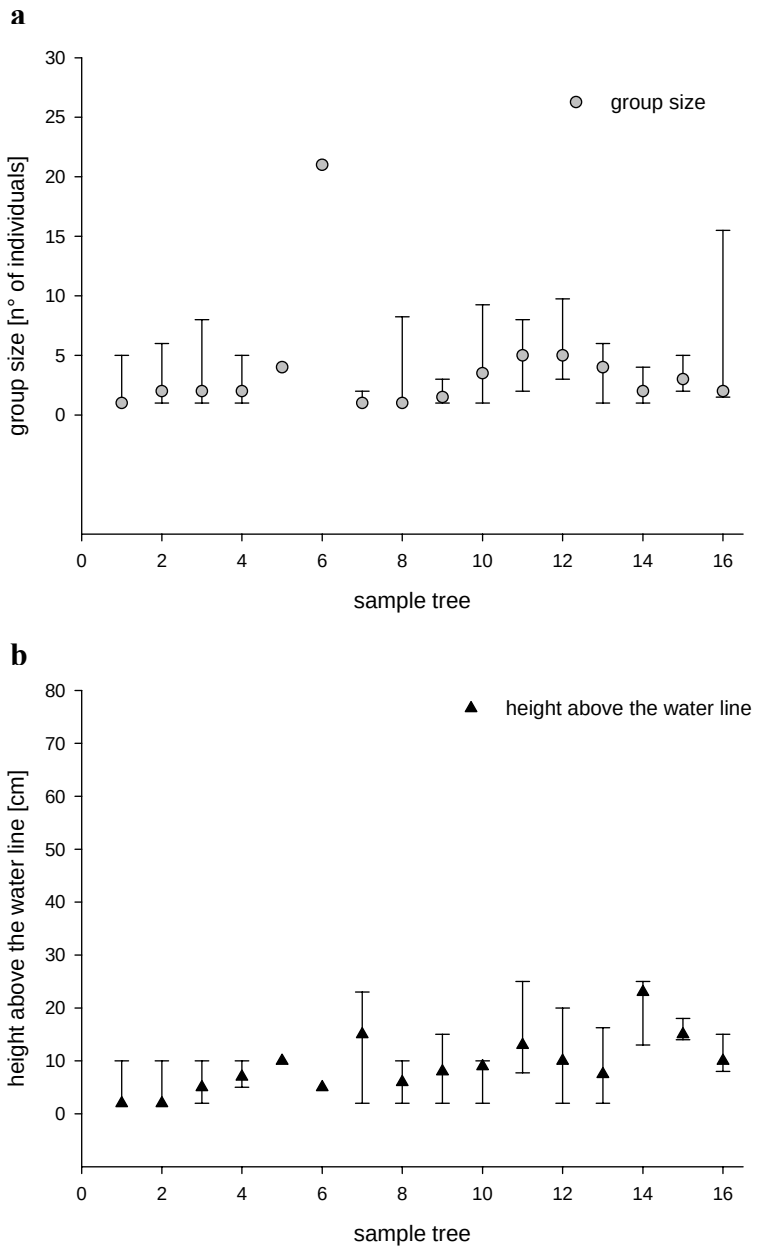

Figure 23a-b. Variation in group size and position of aggregated *P. obliterata* on different sampled tree trunks on Marchantaria Island during the aquatic phases in 2002 and 2003. **a.** group size (tree 15: rarely associated with *P. insularis*, once with *C. adisi*). **b.** height above the water line. Total number of groups: N_{total} = 367. Number of groups on different trees (n°) as indicated by indices (N_1-N_{16}): N_1 = 39; N_2 = 27; N_3 = 23; N_4 = 53; N_5 = 1; N_6 = 1; N_7 = 35; N_8 = 12; N_9 = 22; N_{10} = 12; N_{11} = 24; N_{12} =32; N_{13} = 18; N_{14} = 46; N_{15} = 17; N_{16} = 5. Data points represent the median, lower bars indicate the 1st quartile, upper bars the 3rd quartile.

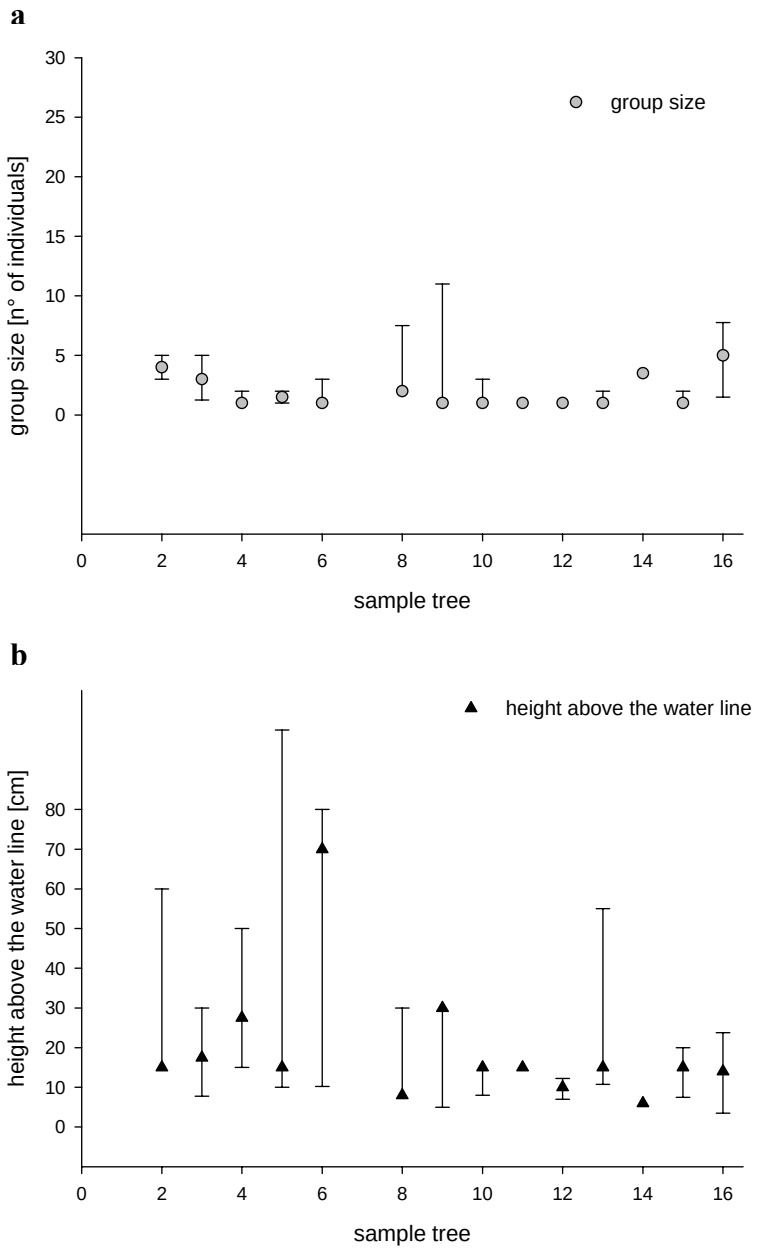

Figure 24a-b. Variation in group size and position of aggregated *P. obliterata* on different sampled tree trunks at Tarumã Mirím River during the aquatic phases in 2002 and 2003. **a.** group size (partly associated with *C. adisi*, rarely with *D. amazonicus*). **b.** height above the water line. Total number of groups: N_{total} = 214. Number of groups on different trees (n°) as indicated by indices (N_2-N_{16}): N_2 = 9; N_3 = 28; N_4 = 50; N_5 = 12; N_6 = 18; N_8 = 12; N_9 = 11; N_{10} = 15; N_{11} = 8; N_{12} =20; N_{13} = 20; N_{14} = 2; N_{15} = 5; N_{16} = 4. Data points represent the median, lower bars indicate the 1st quartile, upper bars the 3rd quartile.

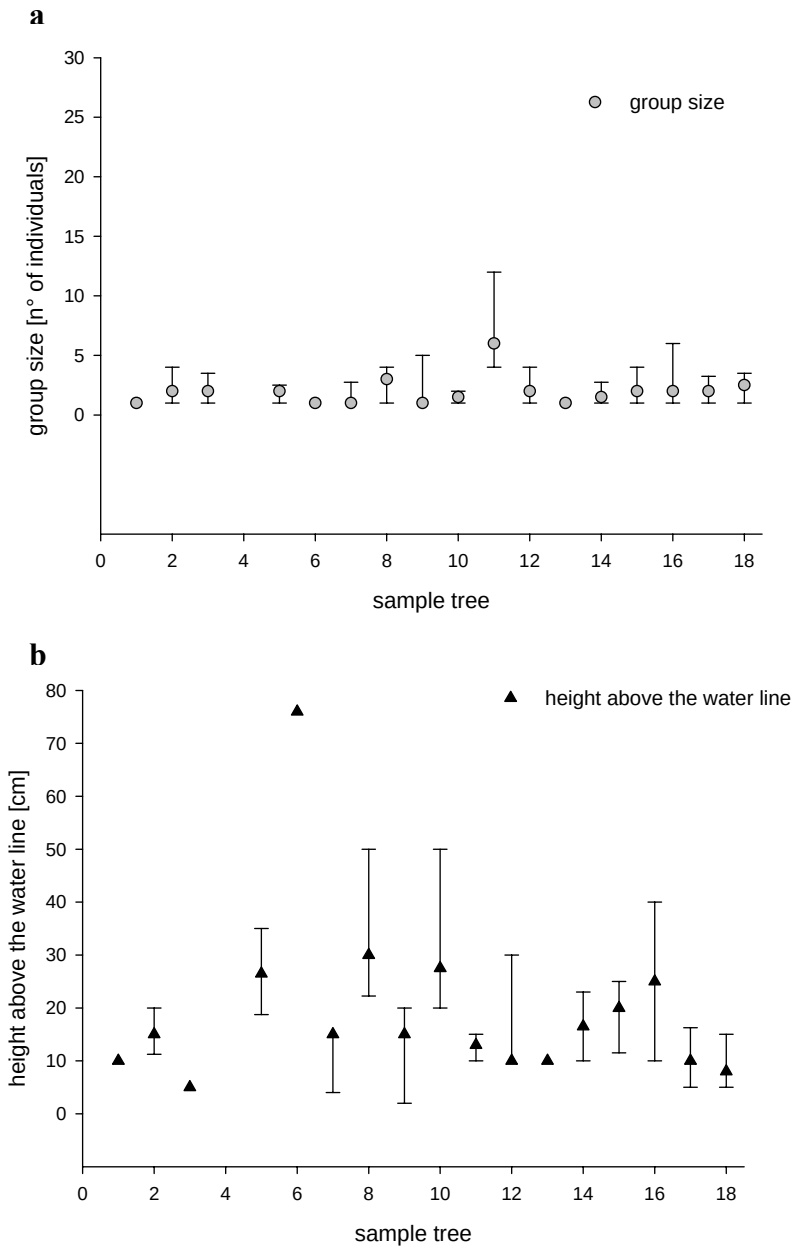

Figure 25a-b. Variation in group size and position of aggregated *P. obliterata* on different sampled tree trunks at Lake Janauarí during the aquatic phases in 2002 and 2003. **a.** group size (partly associated with *P. insularis*, *C. adisi* and *D. amazonicus*). **b.** height above the water line. Total number of groups: N_{total} = 197. Number of groups on different trees (n°) as indicated by indices (N_1-N_{18}): N_1 = 1; N_2 = 15; N_3 = 9; N_5 = 9; N_6 = 2; N_7 = 20; N_8 = 10; N_9 = 3; N_{10} = 16; N_{11} = 14; N_{12} =15; N_{13} = 1; N_{14} = 4; N_{15} = 33; N_{16} = 17; N_{17} = 14; N_{18} = 14. Data points represent the median, lower bars indicate the 1st quartile, upper bars the 3rd quartile.

Kolmogorov-Smirnov tests revealed significant differences in the distributions of both group size ($P < 0.005$) and relative position ($P < 0.001$) of aggregated *P. obliterata* on MA and at TM between 2002 and 2003 (Table 7). The values and variance of aggregation sizes increased from 2002 (MA, mean: 4, median: 2; TM, mean: 3, median: 1) to 2003 (MA, mean: 7, median: 3; TM, mean: 5, median: 2), even though sample sizes were lower in 2003 than in 2002 (Table 8). In contrast, the values and variance of

the height above the water line decreased from 2002 (MA, mean: 13, median: 10; TM, mean: 34, median: 25) to 2003 (MA, mean: 9, median: 8; TM, mean: 12, median: 8). No significant between-year differences were observed for the size (2002, mean: 3, median: 2; 2003, mean: 4, median: 2) and relative position (2002, mean: 20, median: 15; 2003, mean: 21, median: 15) of *P. obliterata* groups at LJ (Table 7), but the sample size was likewise lower in 2003 than in 2002 (Table 8).

Table 7. Statistical parameters of the size and position of *P. obliterata* aggregations, and occurrence of other species within these in three different floodplain forests during the aquatic phases in 2002 and 2003. The sample size (*N*), mean, standard deviation (SD), median, first (Q_1) and third quartiles (Q_3), minimum (X_{min}) and maximum values (X_{max}) and the variance (s^2) are given. Variables that differ significantly between 2002 and 2003 are printed in bold (*, $P < 0.005$; **, $P < 0.001$; Kolmogorov-Smirnov test). Abbreviation: a.w.l., above the water line. Other millipede species: *Poratia insularis* (Pyrgodesmidae), *Cutervodesmus adisi* (Fuhrmannodesmidae), *Docodesmus amazonicus* (Pyrgodesmidae).

	N	Mean	SD	Median	Q_1	Q_3	X_{min}	X_{max}	s^2
Marchantaria Island									
2002									
group size*	251	4	5	2	1	4	1	40	26
height a.w.l.**	251	13	11	10	5	20	2	100	115
P. insularis	8	3	2	2	2	4	1	6	3
C. adisi	1	1	-	1	1	1	1	1	-
2003									
group size*	116	7	16	3	1	7	1	150	261
height a.w.l.**	116	9	9	8	2	10	2	70	75
Tarumã Mirím River									
2002									
group size*	145	3	6	1	1	3	1	50	35
height a.w.l.**	145	34	30	25	15	50	2	200	891
C. adisi	3	28	18	25	11	47	11	47	329
D. amazonicus	2	2	1	2	1	3	1	3	2
2003									
group size*	69	5	7	2	1	6	1	28	45
height a.w.l.**	69	12	11	8	5	15	2	70	125
C. adisi	14	13	7	13	8	19	4	24	42
Lake Janauarí									
2002									
group size	127	3	4	2	1	4	1	22	14
height a.w.l.	127	20	16	15	10	25	2	80	245
P. insularis	27	2	2	2	1	2	1	9	3
D. amazonicus	13	4	2	4	2	5	1	7	3
2003									
group size	70	4	5	2	1	4	1	34	27
height a.w.l.	70	21	23	15	10	25	2	150	513
P. insularis	10	1	0	1	1	1	1	2	0
C. adisi	7	7	3	6	4	9	2	12	11
D. amazonicus	15	5	4	3	3	5	1	17	17

Table 8. Number of individuals (total and single species) in aggregations of *P. obliterata* sampled from trees in three different inundation forests during aquatic phases (2002 and 2003). Millipede species: *P. obliterata* (Pyrgodesmidae), *P. insularis* (Pyrgodesmidae), *C. adisi* (Fuhrmannodesmidae) and *D. amazonicus* (Pyrgodesmidae).

	Total number of specimens	*Poratia obliterata*	*Poratia insularis*	*Cutervodesmus adisi*	*Docodesmus amazonicus*
Marchantaria Island					
2002	927	905	21	1	-
2003	860	860	-	-	-
Tarumã Mirím River					
2002	431	344	-	83	4
2003	357	169	-	188	-
Lake Janauarí					
2002	390	287	57	-	46
2003	262	133	11	46	72

The share of other small millipede species, i.e. *P. insularis*, *C. adisi* and *D. amazonicus*, in groups of *P. obliterata* did not vary significantly between years (Tables 7 & 8). On MA, specimens were associated with *P. insularis* (N = 22 individuals) and *C. adisi* (N = 1) on tree 15 in 2002, but no individuals of any species were found on this respective tree in 2003. Similarly, *D. amazonicus* (N = 4 ind.) only co-occurred with *P. obliterata* at TM in 2002. In contrast, *C. adisi* were solely found associated with *P. obliterata* at LJ in 2003. At both TM and LJ, the comparative share of other species in these aggregations increased from 2002 to 2003.

In 2002, significant median and variance inhomogeneity was observed in both group size and height above the water line in aggregated *P. obliterata* among different inundation forests. Whereas group sizes did not differ between MA and LJ, aggregation sizes at TM were significantly distinct from both MA (P = 0.002) and LJ (P = 0.049). All populations differed significantly in their relative position of aggregations to the water line (P = 0.000). The shortest average distance was recorded for groups on MA, whereas aggregations at LJ and, particularly, those at TM were located at further distances (Table 7).

In 2003, median and variance values of group sizes were homogeneous in all populations, showing no significant difference. In contrast, inhomogeneity in both median and variance values of the height above the water line was revealed among populations. The position of groups did not vary between MA and TM. However, it was significantly distinct at LJ (P = 0.000), as the local aggregated individuals did not

change their relative position from 2002 to 2003, whereas those on MA and TM moved closer to the water line.

The share of other small millipede species, i.e. *P. insularis*, *C. adisi* and *D. amazonicus*, in groups of *P. obliterata* did not vary significantly among the locations, except for the occurrence of *C. adisi* between TM and LJ (P = 0.037), since the species is more abundant at TM (Tables 7 & 8). *P. insularis* co-occurred only at MA and LJ, *C. adisi* mainly at TM and also at LJ, with *D. amazonicus* mainly at LJ and rarely at TM.

At MA, the group size of aggregated *P. obliterata* was positively correlated with residence time in both years (2002: τ = 0.096; 2003: τ = 0.220; Fig. 26a-b), negatively correlated with precipitation in 2003 (τ = -0.271; Fig. 26b), and positively correlated with height above the water line in 2002 (τ = 0.146; Fig. 27a) (Table 9). When omitting the effects of other variables in partial correlations, the relation to residence time was weak (2002: τ_p = 0.049; 2003: τ_p = 0.050). The strongest partial correlation was observed between group size and precipitation in 2003 (τ_p = -0.170), followed by group size and height in 2002 (τ_p = 0.121). The height above the water line was positively correlated with residence time in both years (2002: τ = 0.343; 2003: τ = 0.334; Fig. 28a-b), but negatively correlated with precipitation in 2002 (τ = -0.342; Fig. 28a) (Table 9). When using partial correlations, the magnitude of the relation between height and residence time (τ_p = 0.125) as well as precipitation (τ_p = -0.138) in 2002 was almost equal. The occurrence of *P. insularis* within the aggregations was not related to any of the variables.

At TM, aggregation sizes were positively correlated with residence time (τ = 0.188), but negatively correlated with precipitation (τ = -0.254) in 2003 (Fig. 29b; Table 9). Partial correlation coefficients revealed a stronger relation between group size and precipitation (τ_p = -0.232) than between group size and residence time (τ_p = 0.155) in 2003. Aggregation sizes are not correlated with the height above the water line (Fig. 30a-b).The relative height was negatively correlated with residence time (τ = -0.149), but positively correlated with precipitation (τ = 0.226) in 2002 (Fig. 31a). Partial correlations showed a weak correlation of height with residence time (τ_p = 0.050) and a stronger relation between height and precipitation (τ_p = 0.178) in 2002. The share of *C. adisi* within the aggregations was positively correlated with residence time (τ_p = 0.407) in 2003.

At LJ, group size was positively correlated with residence time (τ = 0.178), but negatively correlated with precipitation (τ = -0.194) in 2003 (Fig. 32b; Table 9.). Partial

correlations revealed a weak correlation with residence time (τ_p = 0.067) and a stronger relation between group size and precipitation (τ_p = -0.102) in 2003. Aggregation size was not correlated with height above the water line (Fig. 33a-b). The relative height was positively correlated with residence time in both years (2002: τ = 0.237; 2003: τ = 0.228; Fig. 34a-b), but negatively correlated with precipitation in 2002 (τ = -0.157; Fig. 34a). Using partial correlations, the relation between height and residence time (τ_p = 0.179) remained distinct, while there was no correlation observed between height and precipitation (τ_p = -0.001) in 2002. The occurrence of other species within these aggregations was positively correlated with residence time (τ = 0.458), negatively correlated with precipitation (τ = -0.392) in 2003, and also negatively correlated with height above the water line in 2002 (τ = -0.249), but positively correlated with height in 2003 (τ = 0.272). Partial correlation coefficients revealed relations between the presence of other species and residence time (τ_p = 0.243), height (τ_p = 0.207), and precipitation (τ_p = -0.152) in 2003.

Omitting extreme sample data did not modify the significance level of any relation observed in the correlation tests.

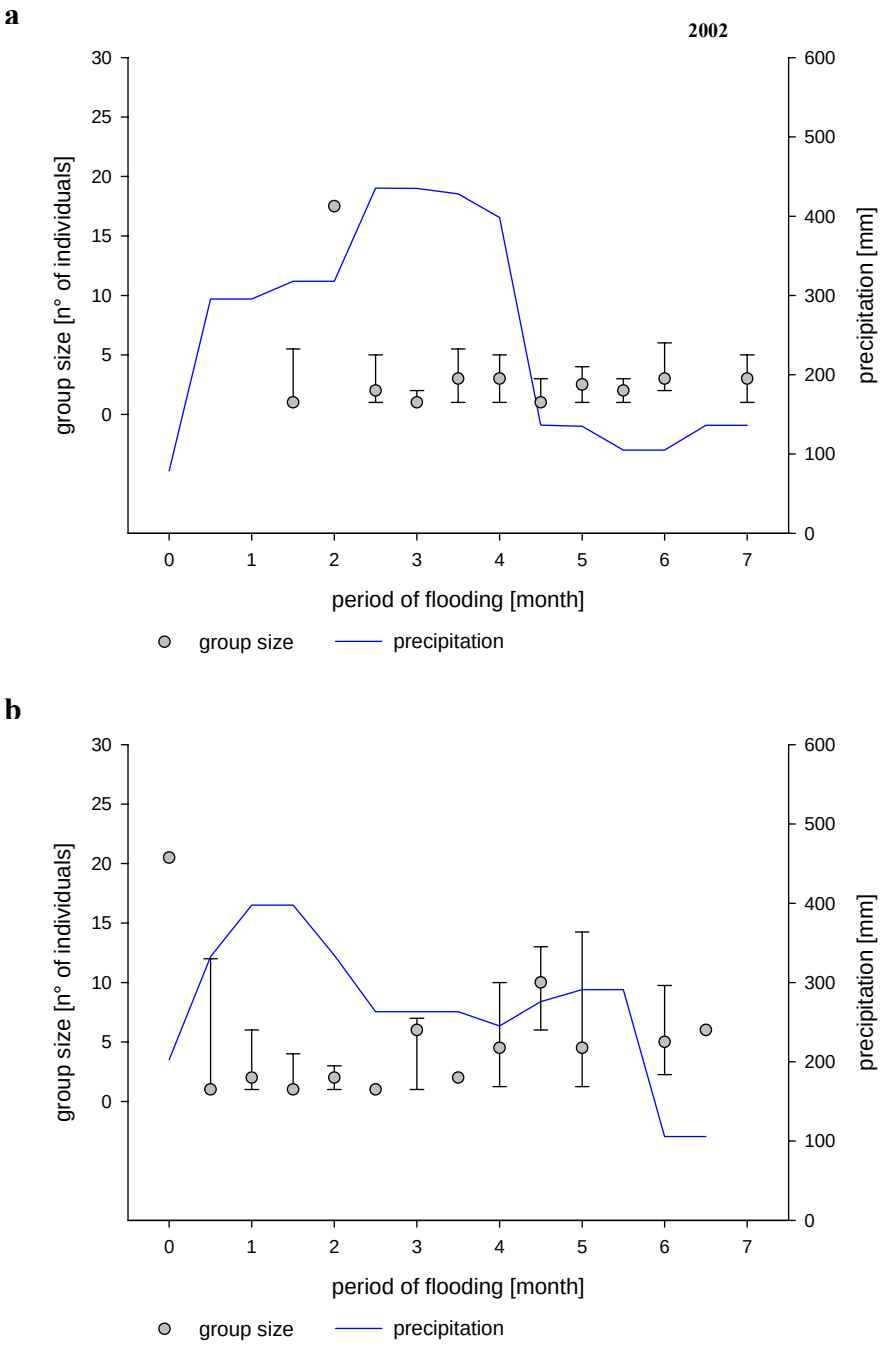

Figure 26a-b. Relation between group sizes in aggregated *P. obliterata* (rarely associated with *P. insularis*, once with *C. adisi*) on tree trunks at Marchantaria Island and regional precipitation during the aquatic phase. **a.** 2002. Total number of groups: N_{total} = 251. Number of groups per month of inundation as indicated by indices ($N_{1.5}$-N_7): $N_{1.5}$ = 18; N_2 = 2; $N_{2.5}$ = 17; N_3 = 19; $N_{3.5}$ = 10; N_4 = 39; $N_{4.5}$ = 51; N_5 = 40; $N_{5.5}$ =19; N_6 = 27; N_7 = 9. **b.** 2003. N_{total} = 116. Number of groups per month of inundation (N_0-$N_{6.5}$): N_0 = 2; $N_{0.5}$ = 3; N_1 = 21; $N_{1.5}$ = 8; N_2 = 17; $N_{2.5}$ = 1; N_3 = 15; $N_{3.5}$ = 1; N_4 = 20; $N_{4.5}$ = 3; N_5 = 12; N_6 = 12; $N_{6.5}$ = 1. Data points represent the median, lower bars indicate the 1st quartile, upper bars the 3rd quartile. Levels of significance for correlations are given in Table 9.

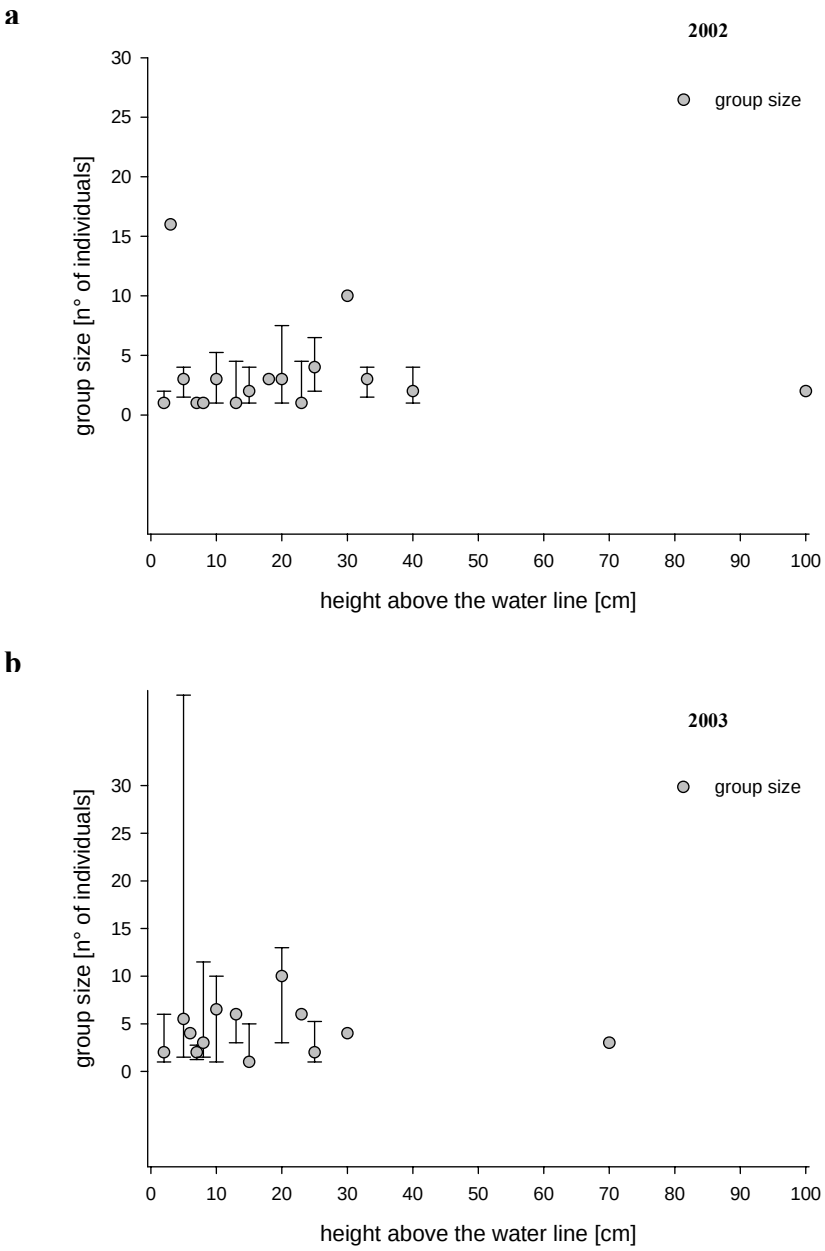

Figure 27a-b. Relation between group size (rarely associated with *P. insularis*) and position of aggregated *P. obliterata* on tree trunks at Marchantaria Island during the aquatic phase. **a.** 2002. Total number of groups: N_{total} = 251. Number of groups in different heights (in cm) as indicated by indices (N_2-N_{100}): N_2 = 54; N_3 = 1; N_5 = 21; N_7 = 17; N_8 = 3; N_{10} = 30; N_{13} = 25; N_{15} = 28; N_{18} = 2; N_{20} = 13; N_{23} = 20; N_{25} = 22; N_{30} = 2; N_{33} = 9; N_{40} = 3; N_{100} =1. **b.** 2003. N_{total} = 116. Number of groups in different heights (N_2-N_{70}): N_2 = 47; N_5 = 4; N_6 = 1; N_7 = 4; N_8 = 5; N_{10} = 30; N_{13} = 4; N_{15} = 11; N_{20} = 3; N_{23} = 1; N_{25} = 4; N_{30} = 1; N_{70} = 1. One extreme sample (group size: 150 individuals; height: 13 cm) was omitted from illustration. Data points represent the median, lower bars indicate the 1st quartile, upper bars the 3rd quartile. Levels of significance for correlations are given in Table 9.

Table 9. Correlation matrix for size, position and share of foreign specimens in aggregations of *P. obliterata*, with residence time on tree trunks and precipitation levels in three different inundation forests during aquatic phases (2002 and 2003). Kendall`s correlation coefficients τ and *P*-values (n.s. = $P < 0.05$) are listed. Partial correlation coefficients (significant interactions between variables omitted) are given in brackets. Significant correlations are printed in bold. Abbreviation: a.w.l., above the water line.

Marchantaria Island			
2002	residence time	precipitation	height a.w.l.
group size	**0.096 (0.049)** **0.024**	-0.0518 n.s.	**0.146 (0.121)** **0.000**
height a.w.l.	**0,343 (0.125)** **0.000**	**-0.342 (-0.138)** **0.000**	
P. insularis	0.372 n.s.	-0.465 n.s.	0.546 n.s.
2003			
group size	**0.220 (0.050)** **0.000**	**-0.271 (-0.170)** **0.000**	0.046 n.s.
height a.w.l.	**0.334** **0.000**	-0.116 n.s.	
Tarumã Mirím River			
2002	residence time	precipitation	height a.w.l.
group size	0.066 n.s.	0.016 n.s.	-0.016 n.s.
height a.w.l.	**-0.149 (0.050)** **0.008**	**0.226 (0.178)** **0.000**	
other millipede species	-0.105 n.s.	0.000 n.s.	0.105 n.s.
2003			
group size	**0.188 (0.155)** **0.023**	**-0.254 (-0.232)** **0.002**	0.069 n.s.
height a.w.l.	0.102 n.s.	0.014 n.s.	
C. adisi	**0.407** **0.042**	-0.075 n.s.	0.035 n.s.
Lake Janauarí			
2002	residence time	precipitation	height a.w.l.
group size	0.029 n.s.	-0.106 n.s.	0.019 n.s.
height a.w.l.	**0.237 (0.179)** **0.000**	**-0.157 (-0.001)** **0.008**	
other millipede species	-0.048 n.s.	0.047 n.s.	**-0.249** **0.038**
2003			
group size	**0.178 (0.067)** **0.029**	**-0.194 (-0.102)** **0.018**	0.063 n.s.
height a.w.l.	**0.228** **0.004**	-0.088 n.s.	
other millipede species	**0.458 (0.243)** **0.000**	**-0.392 (-0.151)** **0.004**	**0.272 (0.207)** **0.047**

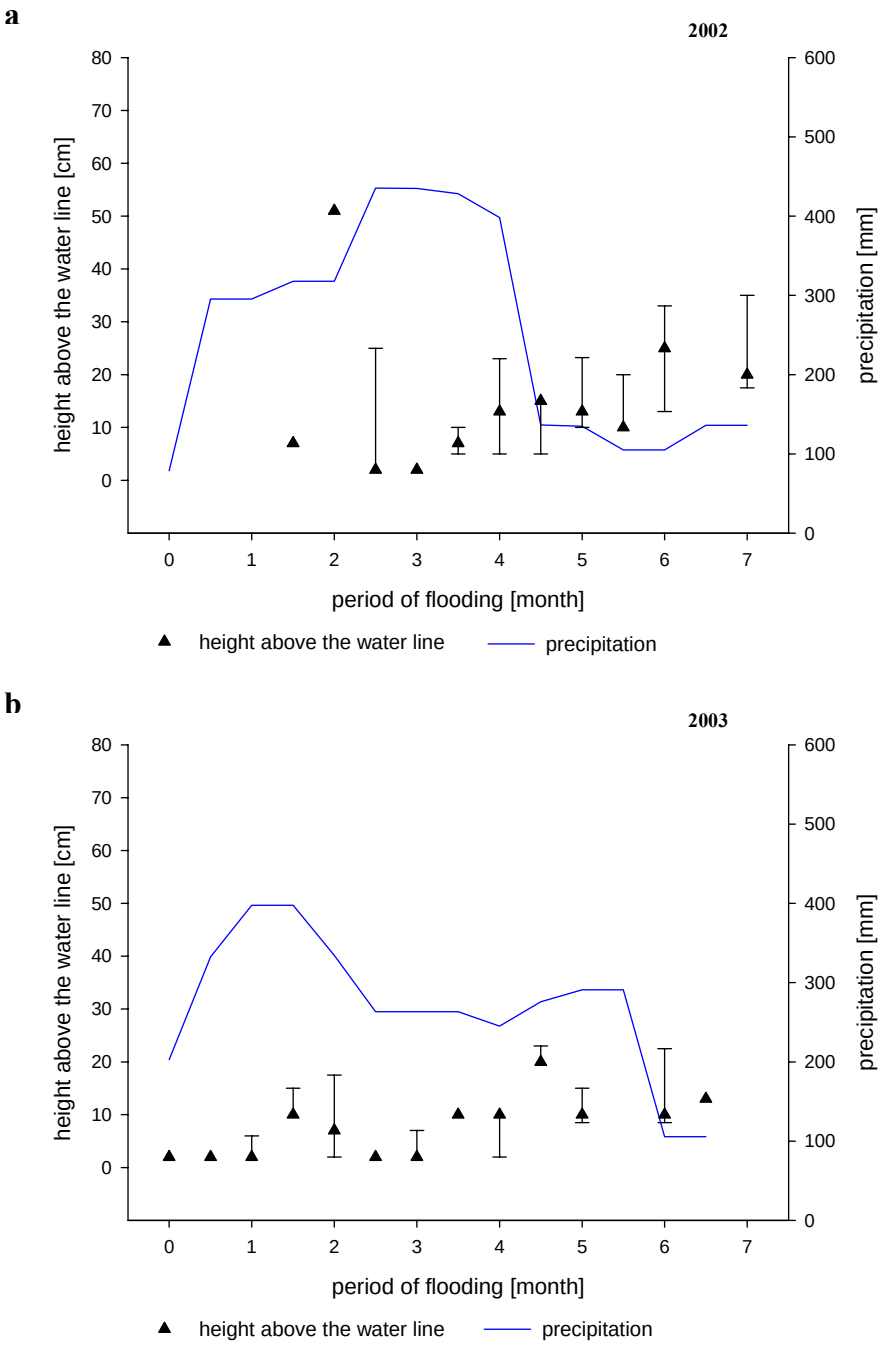

Figure 28a-b. Relation between the position of aggregated *P. obliterata* groups on tree trunks on Marchantaria Island and regional precipitation during the aquatic phase. **a.** 2002. **b.** 2003. For *N* see Figure 26. Data points represent the median, lower bars indicate the 1st quartile, upper bars the 3rd quartile. Levels of significance for correlations are given in Table 9.

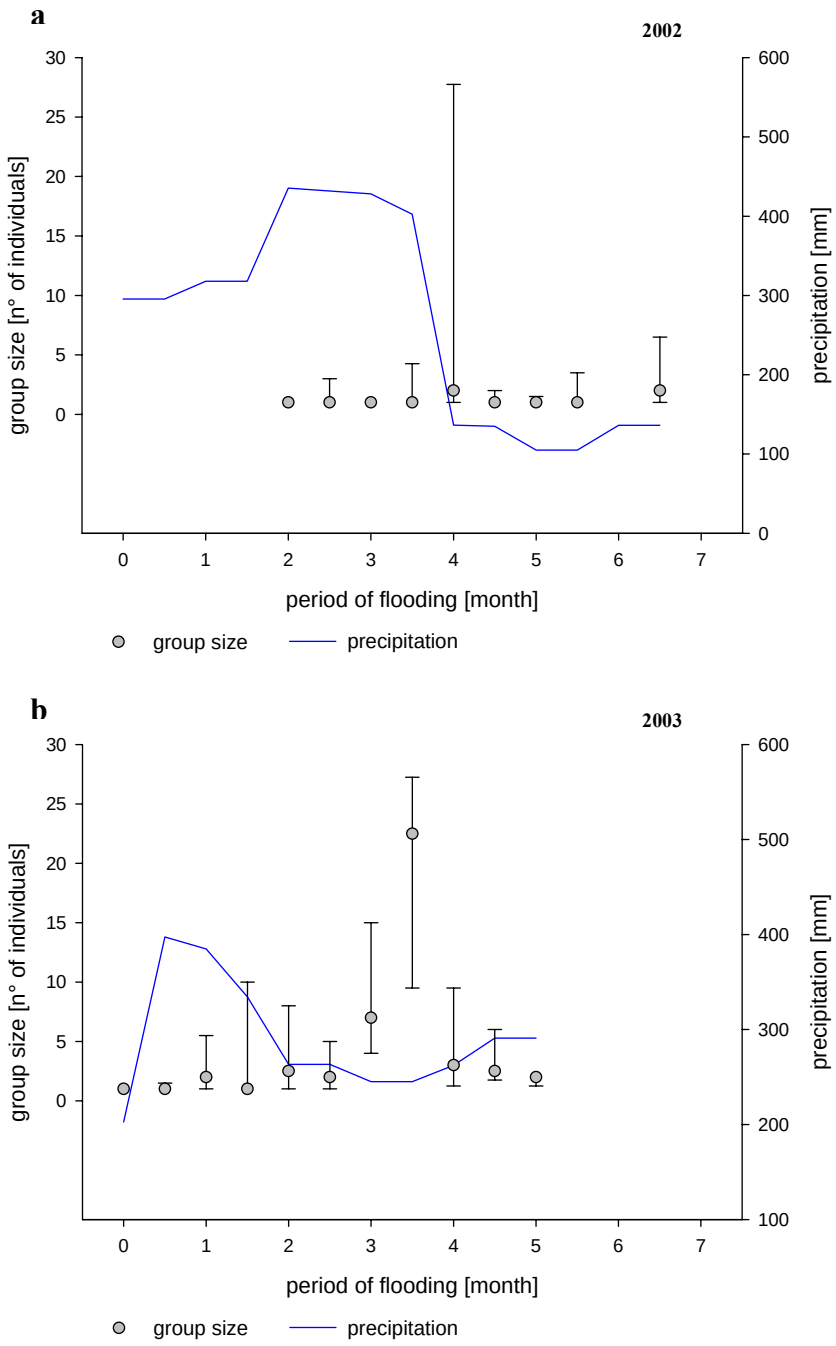

Figure 29a-b. Relation between group sizes in aggregated *P. obliterata* (partly associated with *C. adisi*, rarely with *D. amazonicus*) on tree trunks at Tarumã Mirím River and regional precipitation during the aquatic phase. **a.** 2002. Total number of groups: N_{total} = 145. Number of groups per month of inundation as indicated by indices (N_2-$N_{6.5}$): N_2 = 8; $N_{2.5}$ = 30; N_3 = 16; $N_{3.5}$ = 34; N_4 = 4; $N_{4.5}$ = 22; N_5 = 13; $N_{5.5}$ = 5; $N_{6.5}$ = 13. **b.** 2003. N_{total} = 69. Number of groups per month of inundation (N_0-N_5): N_0 = 1; $N_{0.5}$ = 5; N_1 = 5; $N_{1.5}$ = 4; N_2 = 6; $N_{2.5}$ = 15; N_3 = 3; $N_{3.5}$ = 4; N_4 = 8; $N_{4.5}$ = 10; N_5 = 8. Data points represent the median, lower bars indicate the 1st quartile, upper bars the 3rd quartile. Levels of significance for correlations are given in Table 9.

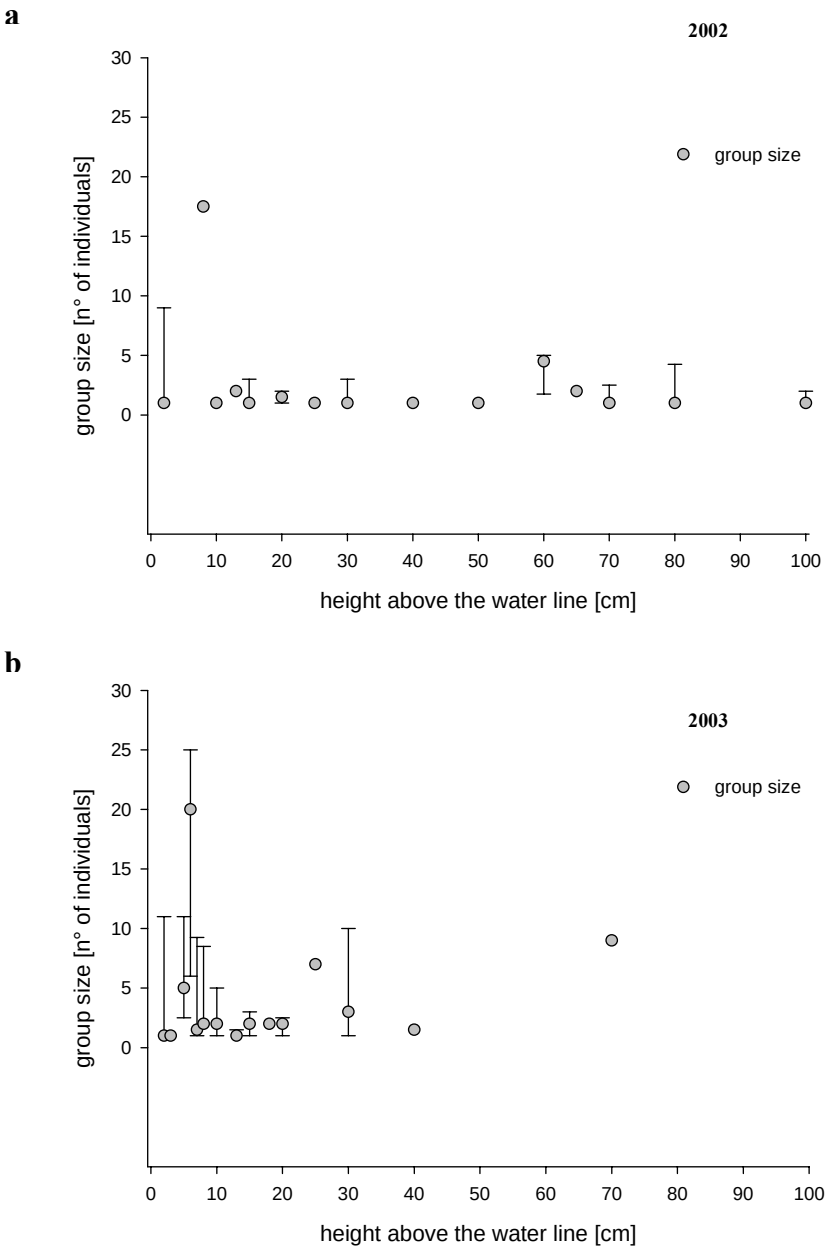

Figure 30a-b. Relation between group size (partly associated with *C. adisi*, rarely with *D. amazonicus*) and position of aggregated *P. obliterata* on tree trunks at Tarumã Mirím River during the aquatic phase. **a.** 2002. Total number of groups: N_{total} = 145. Number of groups in different heights (in cm) as indicated by indices (N_2-N_{200}): N_2 = 7; N_8 = 2; N_{10} = 23; N_{13} = 1; N_{15} = 28; N_{18} = 1; N_{20} = 4; N_{23} =1; N_{25} = 7; N_{30} =24; N_{40} = 4; N_{50} = 13; N_{60} = 6; N_{65} = 1; N_{70} = 8; N_{80} = 6; N_{100} = 8; N_{200} = 1. One extreme sample (N_{200}) was omitted from illustration. **b.** 2003. N_{total} = 69. Number of groups in different heights (N_2-N_{70}): N_2 = 11; N_3 = 1; N_5 = 9; N_6 = 3; N_7 = 6; N_8 = 6; N_{10} = 7; N_{13} = 5; N_{15} = 8; N_{18} = 1; N_{20} = 5; N_{25} = 1; N_{30} = 3; N_{40} = 2; N_{70} = 1. Data points represent the median, lower bars indicate the 1st quartile, upper bars the 3rd quartile. Levels of significance for correlations are given in Table 9.

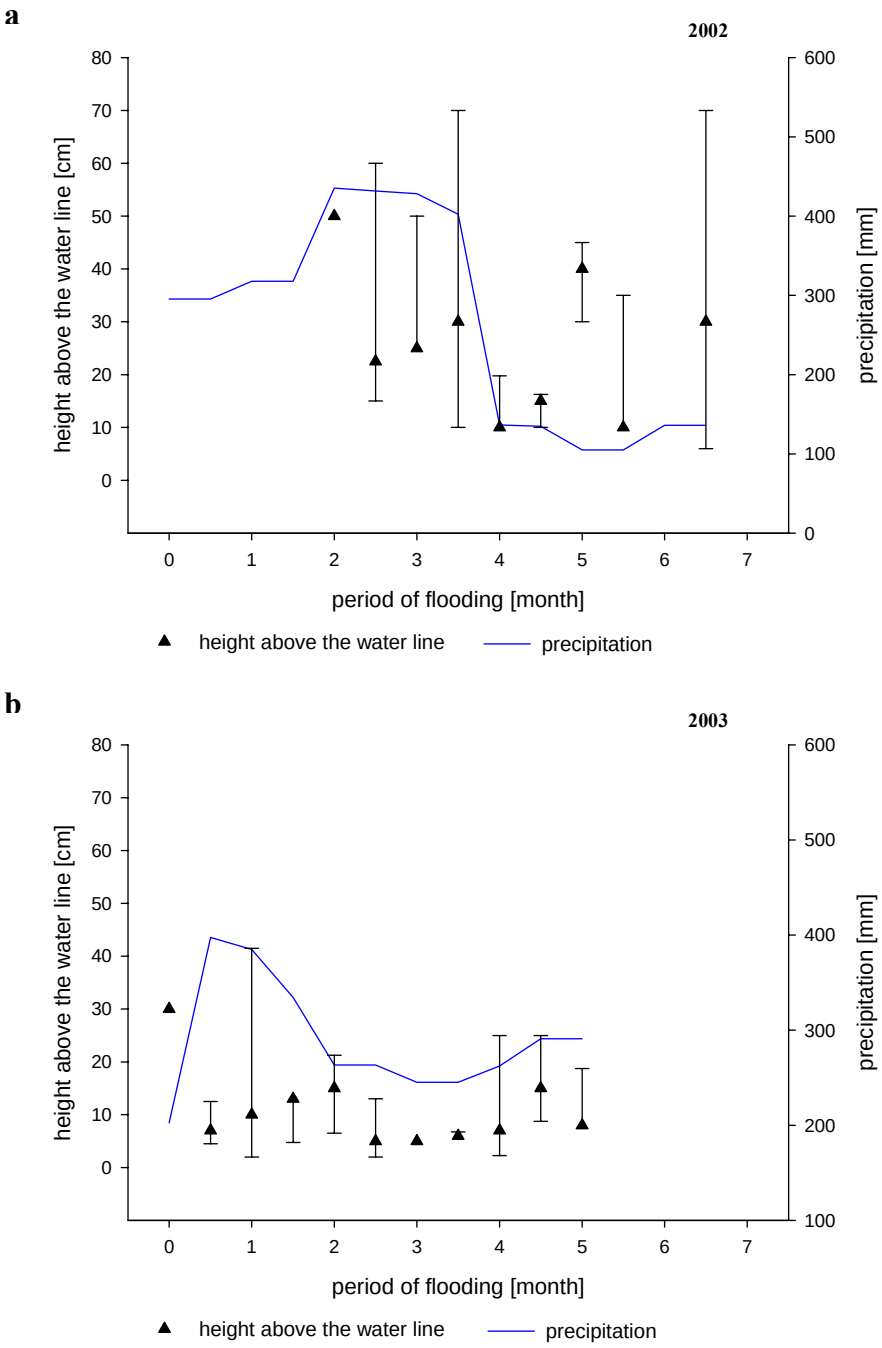

Figure 31a-b. Relation between the position of aggregated *P. obliterata* groups on tree trunks at Tarumã Mirím River and regional precipitation during the aquatic phase. **a.** 2002. **b.** 2003. For *N* see Figure 29. Data points represent the median, lower bars indicate the 1st quartile, upper bars the 3rd quartile. Levels of significance for correlations are given in Table 9.

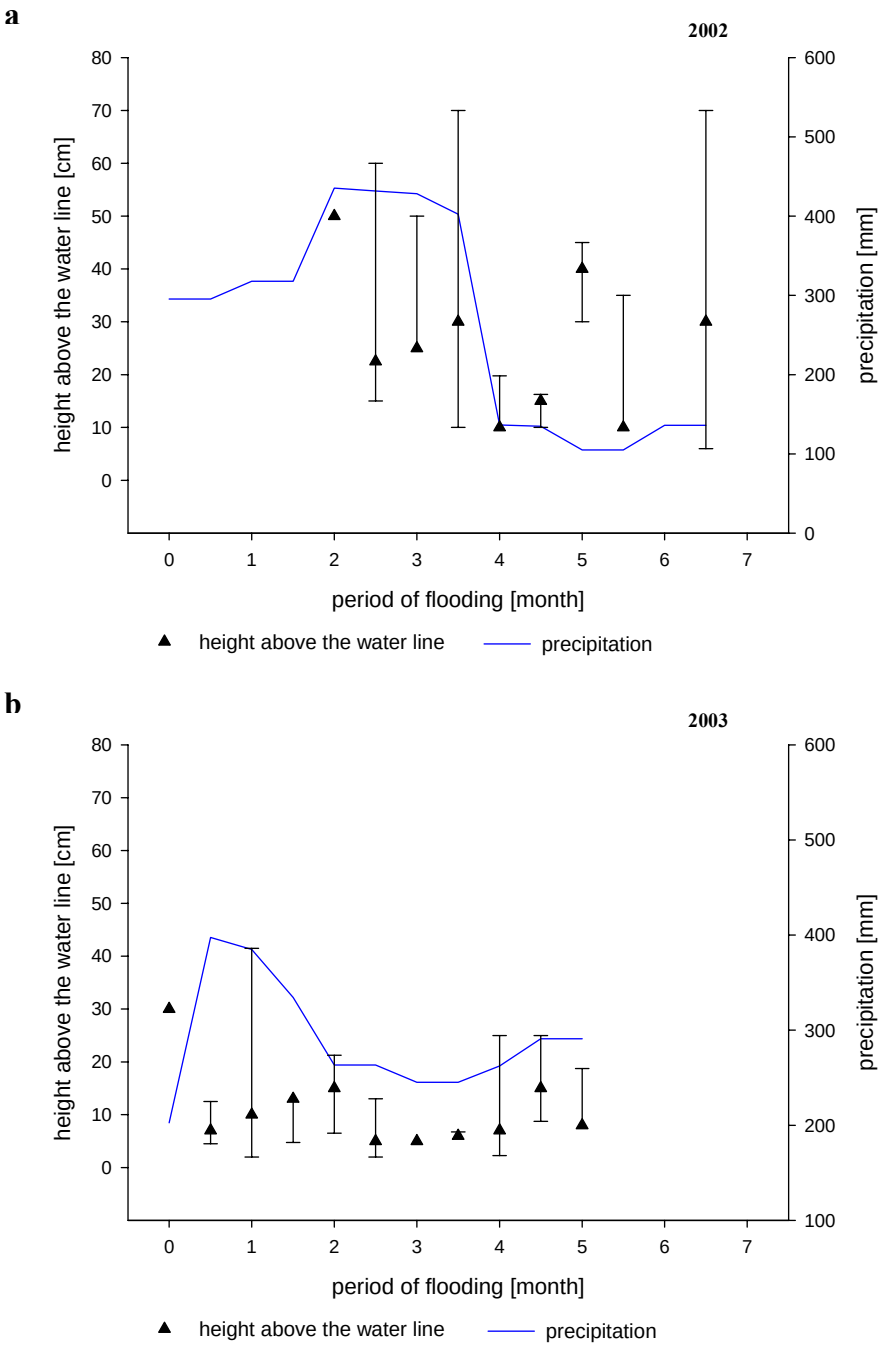

Figure 31a-b. Relation between the position of aggregated *P. obliterata* groups on tree trunks at Tarumã Mirím River and regional precipitation during the aquatic phase. **a.** 2002. **b.** 2003. For *N* see Figure 29. Data points represent the median, lower bars indicate the 1st quartile, upper bars the 3rd quartile. Levels of significance for correlations are given in Table 9.

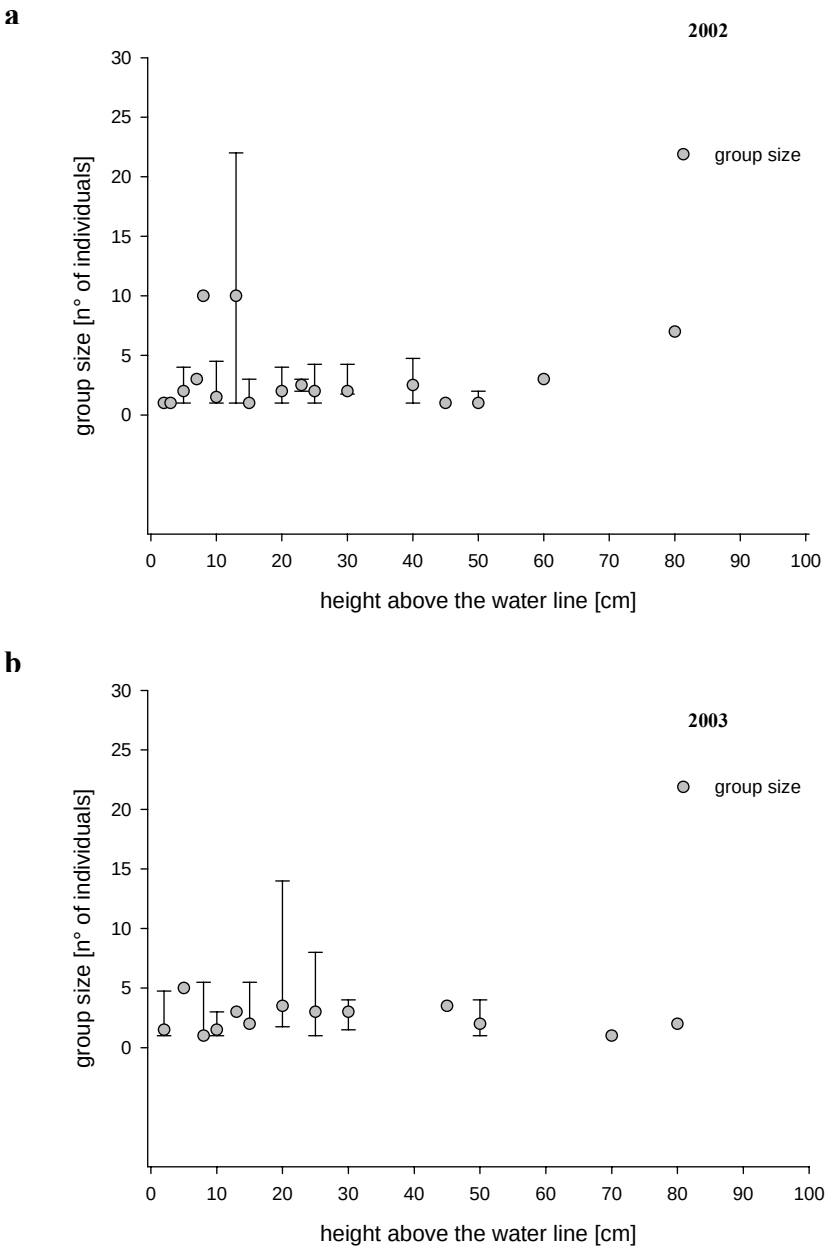

Figure 33a-b. Relation between group size (partly associated with other species) and position of aggregated *P. obliterata* on tree trunks at Lake Janauarí during the aquatic phase. **a.** 2002. Total number of groups: N_{total} = 127. Number of groups in different heights (in cm) as indicated by indices (N_2-N_{80}): N_2 = 6; N_3 = 1; N_5 = 15; N_7 = 2; N_8 = 1; N_{10} = 18; N_{13} = 3; N_{15} = 22; N_{20} = 17; N_{23} = 4; N_{25} = 8; N_{30} = 10; N_{40} = 4; N_{45} = 2; N_{50} = 11; N_{60} = 1; N_{80} = 2. Total numbers of individuals: *P. obliterata*, 287; *P. insularis*, 57; *D. amazonicus*, 46. **b.** 2003. N_{total} = 70. Number of groups in different heights (in cm) (N_2-N_{150}): N_2 = 10; N_5 = 2; N_8 = 4; N_{10} = 16; N_{13} = 2; N_{15} = 9; N_{20} = 6; N_{25} = 7; N_{30} = 5; N_{45} = 2; N_{50} = 3; N_{70} = 1; N_{80} = 2; N_{150} = 1. One extreme sample (N_{150}) was omitted from illustration. Data points represent the median, lower bars indicate the 1st quartile, upper bars the 3rd quartile. Levels of significance for correlations are given in Table 9.

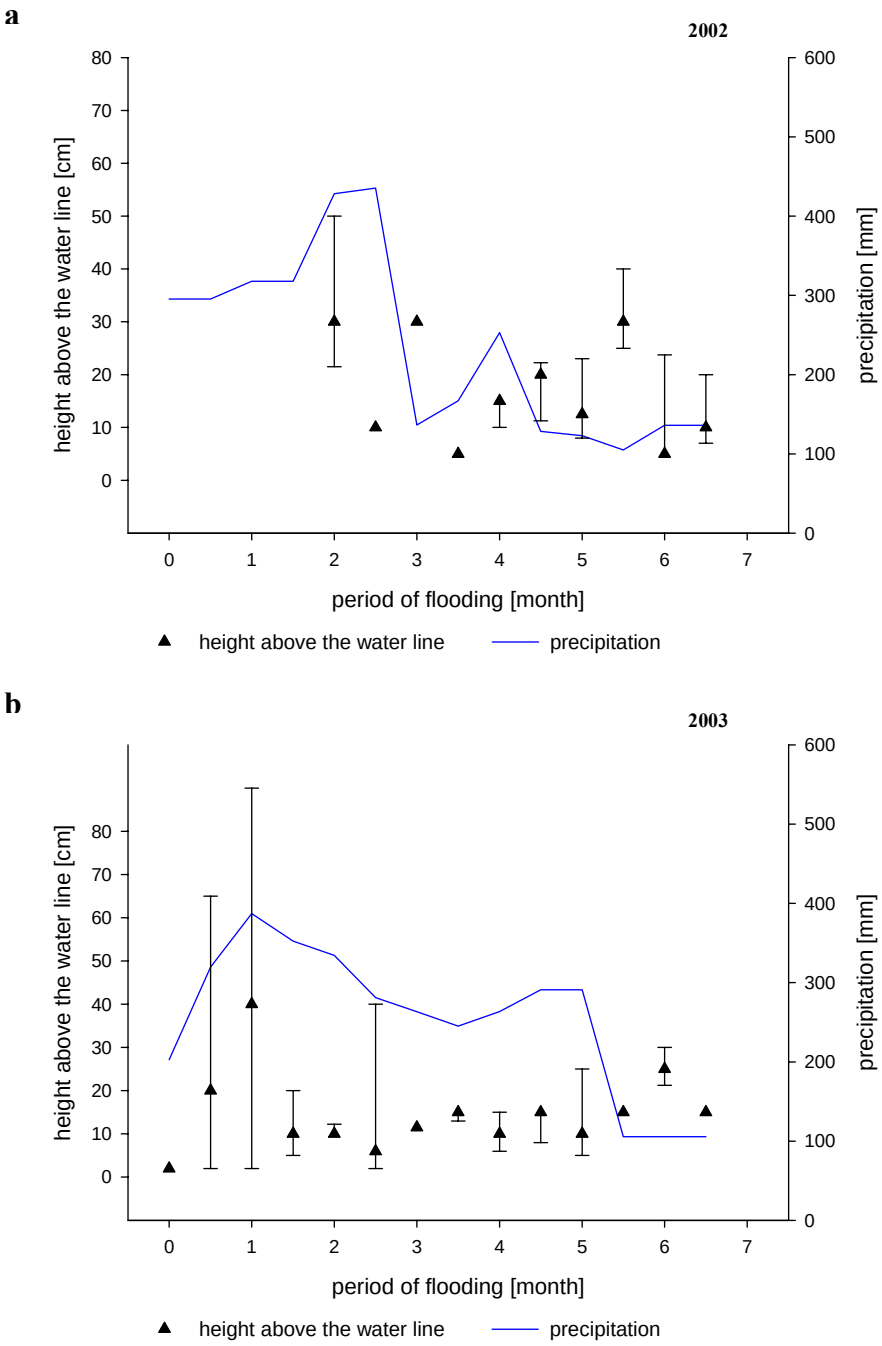

Figure 34a-b. Relation between the position of aggregated *P. obliterata* groups on tree trunks at Lake Janauarí and regional precipitation during the aquatic phase. **a.** 2002. **b.** 2003. For *N* see Figure 32. Data points represent the median, lower bars indicate the 1st quartile, upper bars the 3rd quartile. Levels of significance for correlations are given in Table 9.

3.2.3.2 Banana Plantation

On the upland banana plantation, *P. obliterata* dwelled in the interior of humid pseudo-trunks left on the ground after cutting. Whereas fresh plant material was not accepted by these millipedes, banana stems in an advanced state of decay were, in part, densely populated. *P. obliterata* lived in scattered groups (no body contact) or solitary in the

moist, comb-like chambers of the trunk-forming leaves. When plant material started to desiccate, animals first retreated to still humid, in part rooted, regions underneath the rotten pseudo-stems. Particularly during the drier season, some advanced stages were encountered beneath rotten leaves or pseudo-trunks facing the humid, loamy soil. Along with a drying soil surface millipedes migrated, depending on soil conditions (scanty litter, large fraction of clay), rather horizontally to nearby plant material than vertically into deeper soil layers.

The animals avoided banana stems colonised by ants but did not seem disturbed by the presence of other insects and their larvae or co-occurring millipede species, such as the less common *Myrmecodesmus hastatus* (Bergholz et al. 2004). The total number of individuals sampled during the observations differed little between 2002 and 2003 (Table 10).

Table 10. Number of individuals of *P. obliterata* and co-occurring *M. hastatus* sampled in parallel to aquatic phases (2002 and 2003) on the banana plantation at CPPA/Embrapa.

	Poratia obliterata	*Myrmecodesmus hastatus*
2002	1119	8
2003	1166	18

3.2.4 Phenology

3.2.4.1 Life Cycle (Progress of Stages, Sex Ratio)

3.2.4.1.1 Inundation Forests

Field observations indicate a univoltine life cycle for *P. obliterata* in the inundation forests. At all study sites, i.e. Marchantaria Island, Tarumã Mirím River and Lake Janauarí, the proportion of advanced developmental stages amongst the sampled individuals grew steadily during the aquatic phase, while no recent offspring was detected (Figs 35-37).

In 2002, only few individuals of developmental stages younger than the 5th were found on tree trunks at the beginning of the aquatic phase, i.e. shortly after trunk ascents. The sampled population at Lake Janauarí comprised a higher portion of juvenile stages than those at the other two locations. At all sites, the majority of animals spent the aquatic phase in the 6th and mainly subadult stages, with a fraction of subadults accomplishing adulthood towards the end of inundation. This pattern is most apparent in the population

monitored on Marchantaria Island, where more than 80 % of all individuals encountered during the last month of inundation had developed into adults. Here, Kendall's correlations revealed a significant decrease (τ = -0.733, P = 0.039) in the number of individuals of the 6th stage during the aquatic phase. When data for all inundation forests were pooled, the number of specimens in both 4th and 6th stages declined significantly with time (stage 4: τ = -0.378, P = 0.041; stage 6: τ = -0.458, P = 0.013). Other correlations were not significant. The total number of individuals observed during the aquatic phase did not change significantly.

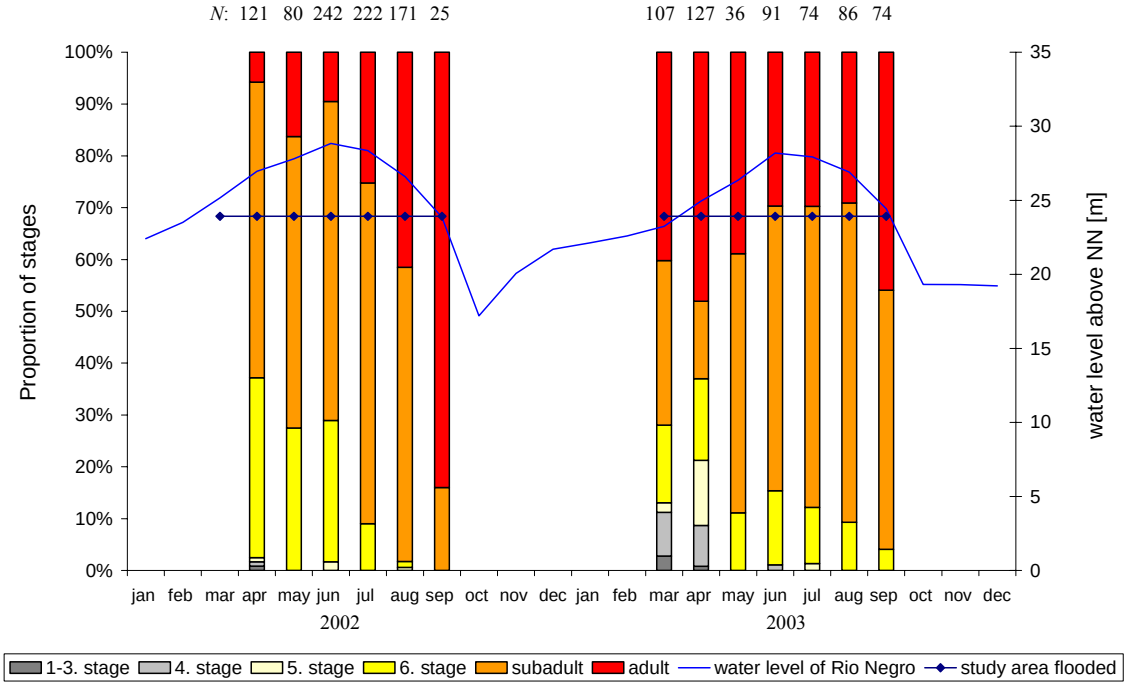

Figure 35. Phenology of *P. obliterata* sampled in the whitewater inundation forest (Várzea) on Marchantaria Island during the aquatic phase in 2002 and 2003.
(Water level of the Negro River: average values between collection dates; total number of individuals (*N*) in 2002: 771, and in 2003: 595; N_{total}: 1366).

In 2003, the proportion of younger developmental stages at the beginning of the aquatic phase was higher than in 2002. That was particularly true for the population observed in the seasonally flooded forest at Lake Janauarí. Whereas age distribution in the last months of flooding resembled that of the previous year for animals from Marchantaria Island and Tarumã Mirím River, the millipedes were perspicuously younger at Lake Janauarí. Here, many 6th and some 5th developmental stages were still encountered near the end of the aquatic phase, most probably representing formerly 3rd and 4th stages at the onset of flooding (April-May). On Marchantaria Island, the number of specimens representing stages 1 to 4 declined significantly with time (stage 1 to 3: τ = -0.724,

P = 0.022; stage 4: τ = -0.620, P = 0.050), while at Tarumã Mirím River, the number of adults increased significantly (τ = 0.966, P = 0.006). When data for all inundation forests were pooled, the number of specimens in the 1st to 4th stage decreased significantly during the aquatic phase (stage 1 to 3: τ = -0.430, P = 0.008; stage 4: τ = -0.423, P = 0.009), while the number of subadults increased significantly (τ = 0.356, P = 0.028). Other correlations were not significant. The total number of individuals observed during the aquatic phase did not alter significantly.

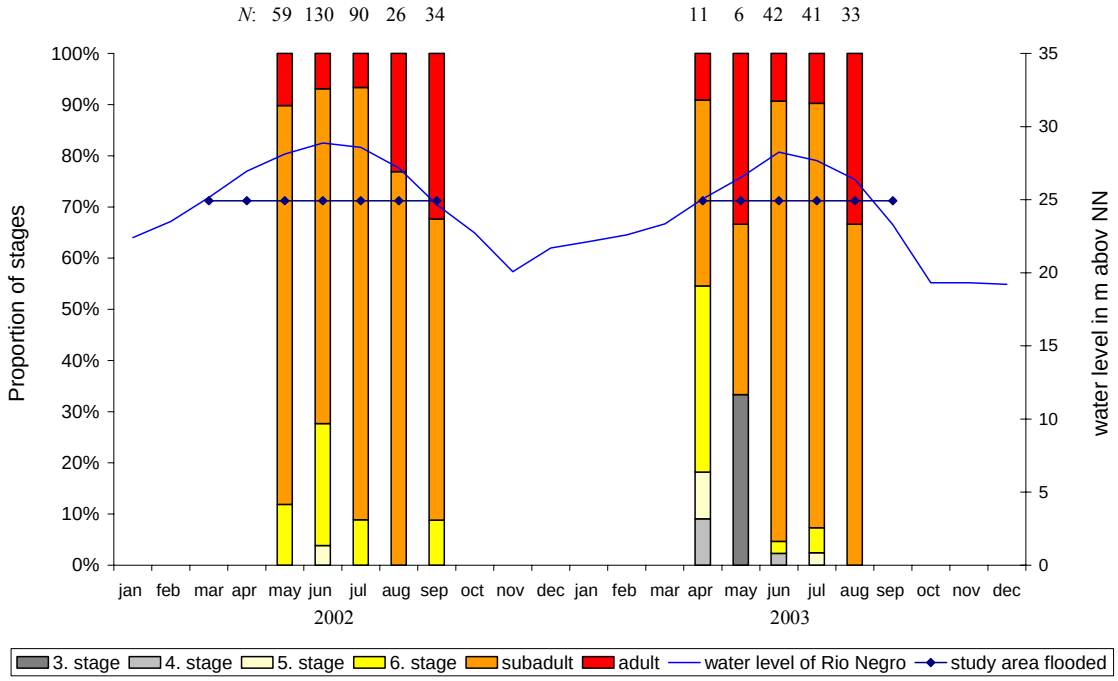

Figure 36. Phenology of *P. obliterata* sampled in the blackwater inundation forest (Igapó) at Tarumã Mirím River during the aquatic phase in 2002 and 2003.
(Water level of the Negro River: average values between collection dates; total number of individuals (*N*) in 2002: 339, and in 2003: 133; N_{total}: 472).

Considering data for both years, the numbers of all juvenile stages declined significantly with time on Marchantaria Island (stage 1 to 3: τ = -0.667, P = 0.002; stage 4: τ = -0.607, P = 0.004; stage 5: τ = -0.417, P = 0.047; stage 6: τ = -0.514, P = 0.015). At Tarumã Mirím River, the number of adults increased significantly during the aquatic phase (τ = 0.713, P = 0.002). If data were pooled for all inundation forest populations, all developmental stages except for stage 6th, subadults and adults declined significantly with time (stage 1 to 3: τ = -0.456, P = 0.001; stage 4: τ = -0.465, P = 0.001; stage 5: τ = -0.316, P = 0.027). Other correlations were not significant. The total number of individuals observed during the aquatic phases did not change significantly.

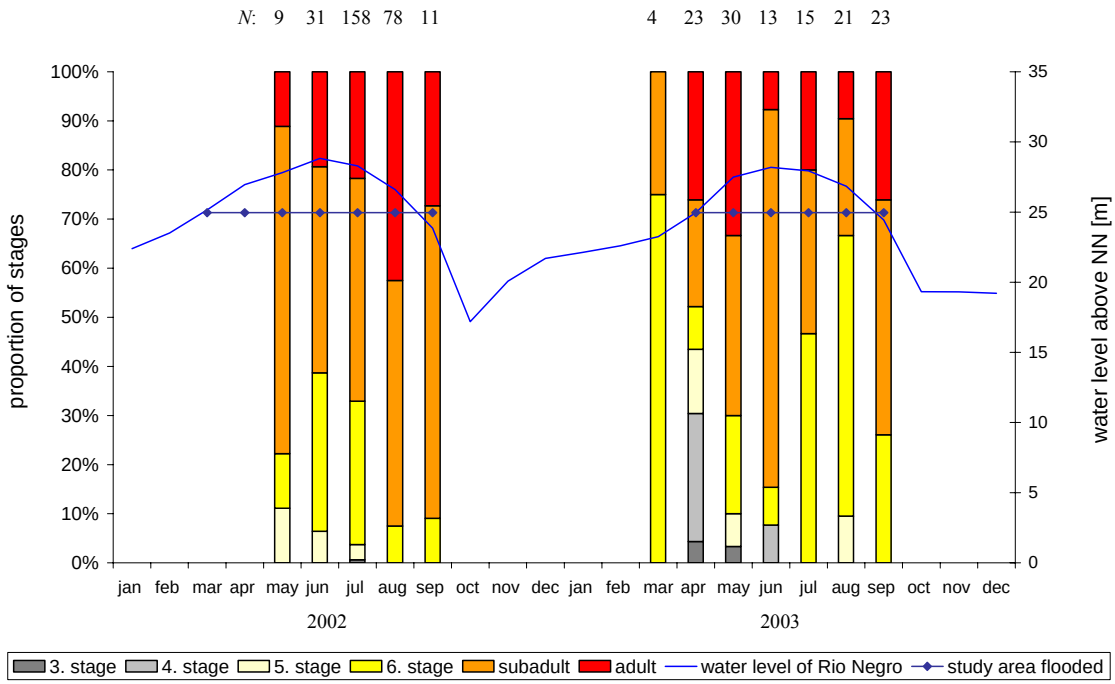

Figure 37. Phenology of *P. obliterata* sampled in the mixedwater inundation forest (Várzea & Igapó) at Lake Janauarí during the aquatic phase in 2002 and 2003.
(Water level of the Negro River: average values between collection dates; total number of individuals (*N*) in 2002: 287, and in 2003: 129; N_{total}: 416).

To study the influence of the aquatic phase on the phenology of *P. obliterata* more precisely, the progress of developmental stages was examined separately for groups of sample trees located at different altitudes, i.e. exposed to flood periods of varying duration. Despite the relatively large variation in flood periods between these groups (up to 40 days on Marchantaria Island; up to 60 days at Tarumã Mirím River; and up to 100 days at Lake Janauarí; Chapter 3.2.1.3), no general differences were observed in the phenology of *P. obliterata*. While the frequencies of immature stages at the onset of the aquatic phase appear to increase with the elevation level of sample trees, the sample sizes per tree group and month are considered too small for evaluation.

The occurrence of female and male individuals (immatures and adults) in all populations studied did not differ significantly in both 2002 and 2003 (Mann-Whitney U test). Consequently, the sex ratio in *P. obliterata* did not differ significantly between years and populations (including the upland population at CPPA/Embrapa; chi-square tests). Both median and variance homogeneity of sex ratio during the aquatic phase was observed for all populations (Kruskal-Wallis test). Average sex ratios (male to female) in 2002 and 2003 accounted for 0.8 and 0.8 on Marchantaria Island; 0.8 and 0.7 at Tarumã Mirím; and 0.6 and 0.7 at Lake Janauarí, respectively (Figs 38-40).

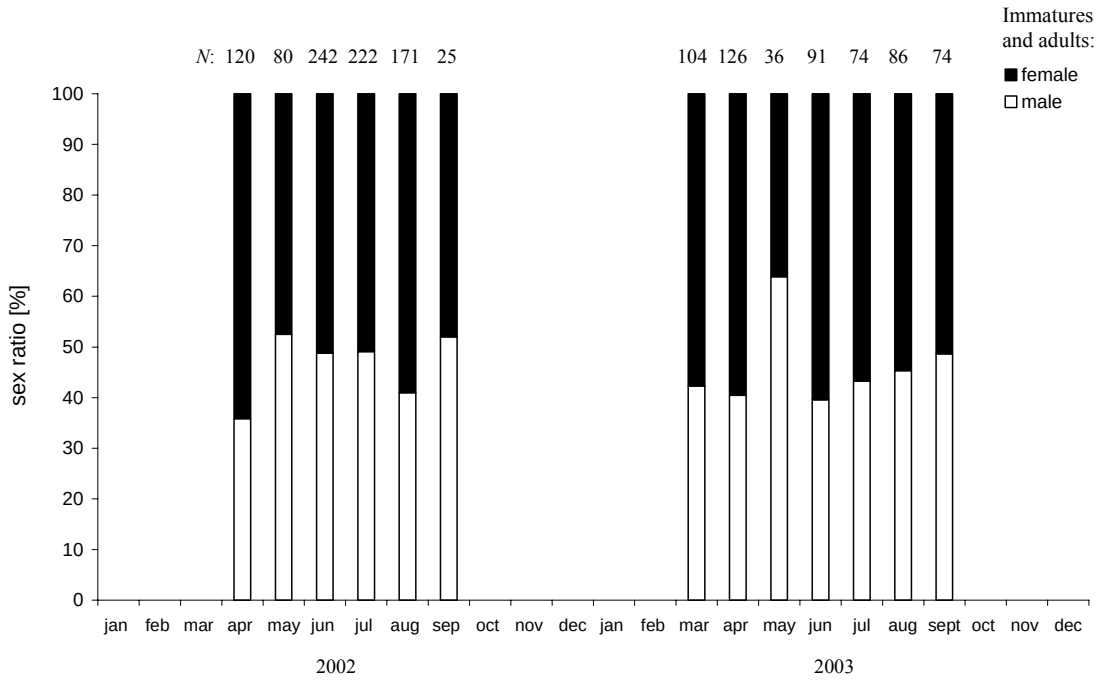

Figure 38. Sex ratio of *P. obliterata* sampled in the whitewater inundation forest (Várzea) on Marchantaria Island during the aquatic phase in 2002 and 2003.
(*N*: number of individuals collected per month; N_{total}: 1361).

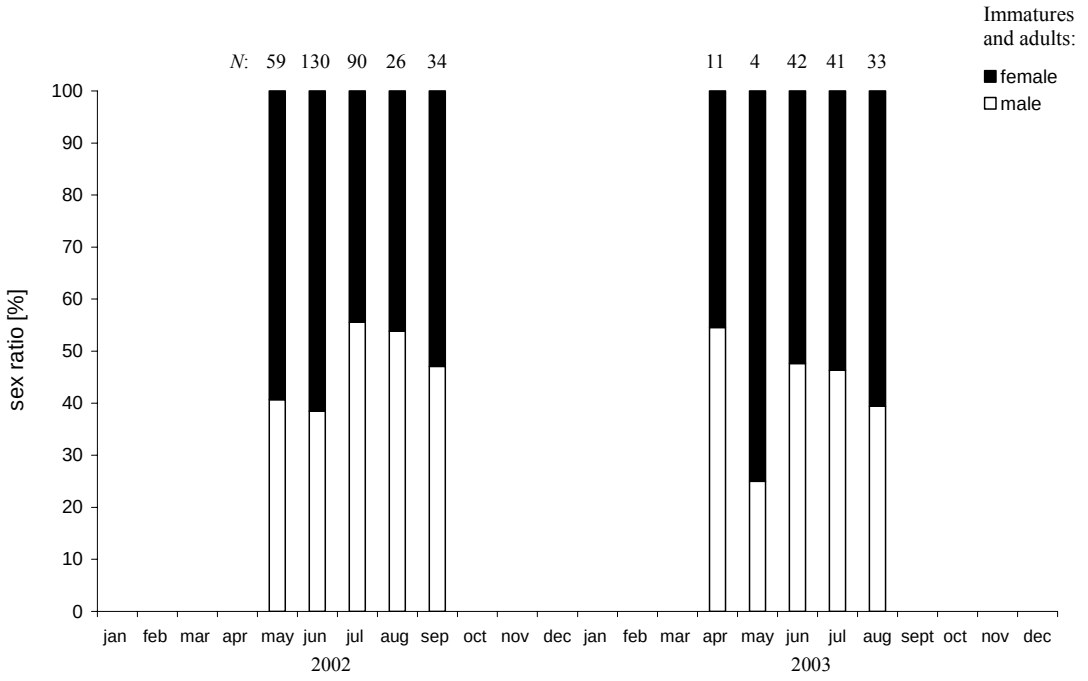

Figure 39. Sex ratio of *P. obliterata* sampled in the blackwater inundation forest (Igapó) at Tarumã Mirím River during the aquatic phase in 2002 and 2003.
(*N*: number of individuals collected per month; N_{total}: 470).

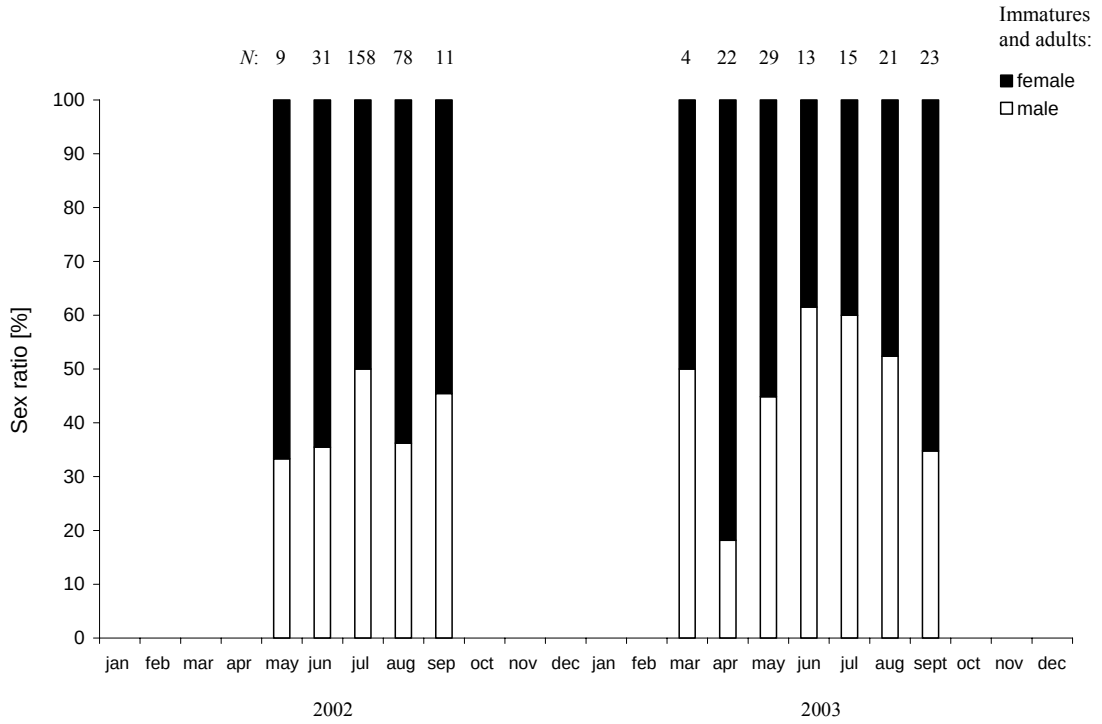

Figure 40. Sex ratio of *P. obliterata* sampled in the mixedwater inundation forest (Várzea & Igapó) at Lake Janauarí during the aquatic phase in 2002 and 2003.
(*N*: number of individuals collected per month; N_{total}: 414).

3.2.4.1.2 Banana Plantation

The population from the upland banana plantation at CPPA/Embrapa showed a plurivoltine life cycle. All developmental stages, i.e. first and advanced juvenile stages and adults, were recorded throughout the observation period, e.g. parallel to the aquatic phase in the inundation forests (Fig. 41).

In 2002, correlations revealed a significant decrease in the total number of individuals collected per month (Kendall's τ = -0.800, P = 0.050). Other correlations were not significant. Despite positive and negative trends, the occurrence of juvenile stages was not significantly correlated with local monthly precipitation or insolation, respectively (Fig. 41).

The frequency of female and male specimens (immatures and adults) did not differ significantly in 2002 and 2003 (Mann-Whitney U test). Consequently, sex ratios of *P. obliterata* did not differ between years (chi-square tests) or months (median and variance homogeneity; Kruskal-Wallis test). Sex ratios (male to female) in 2002 and 2003 averaged 1.0 and 1.1, respectively (Fig. 42).

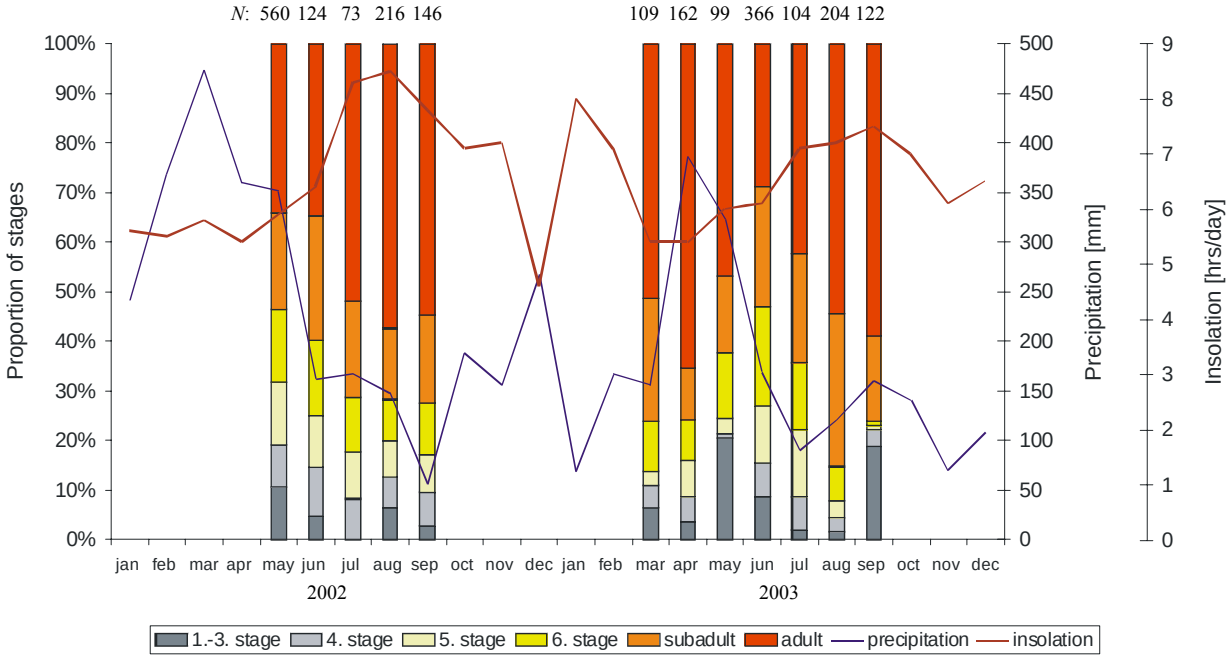

Figure 41. Phenology of *P. obliterata* sampled on the upland banana plantation at CPPA/Embrapa, monitored parallel to the aquatic phases in regional inundation forests in 2002 and 2003. (Number of individuals (*N*) in 2002: 1119, in 2003: 1166; N_{total}: 2285).

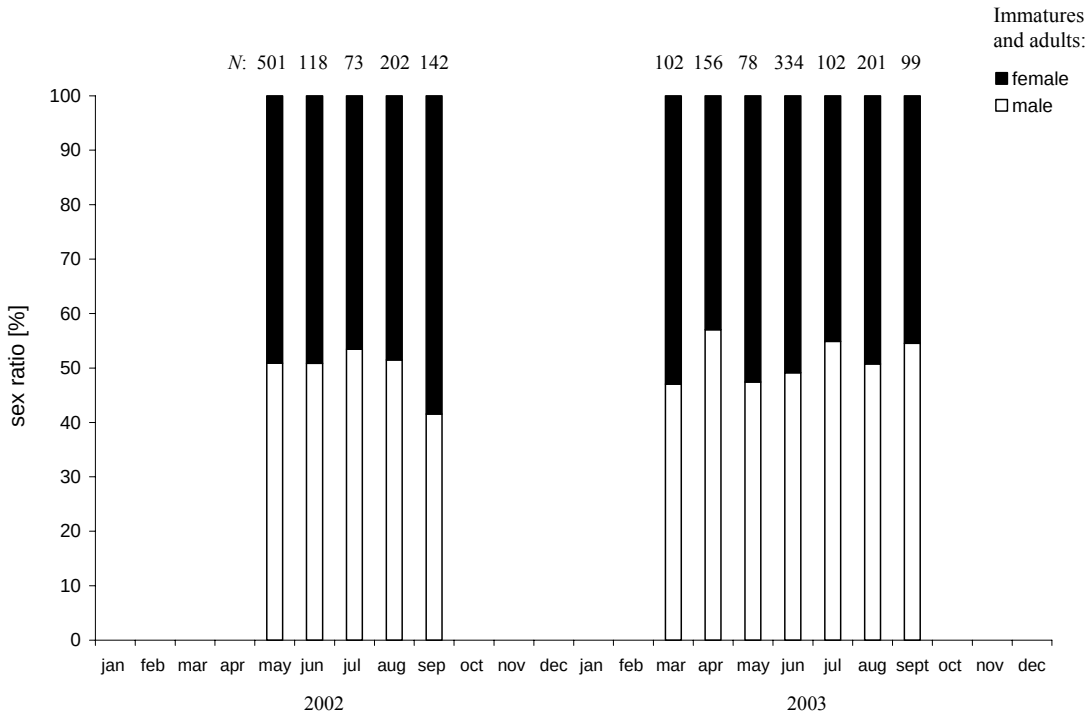

Figure 42. Sex ratio of *P. obliterata* sampled on the upland banana plantation at CPPA/Embrapa, monitored parallel to the aquatic phases in regional inundation forests in 2002 and 2003. (*N*: number of individuals collected per month; N_{total}: 2108).

3.2.4.2 Reproduction (Mating, Eggs)

3.2.4.2.1 Inundation Forests

In the inundation forests, mating was only recorded sporadically at the end of the aquatic phase. On Marchantaria Island (MA), each two copulations were observed in late August (2002 and 2003) and late September (2003). At Tarumã Mirím River, no mating was noted, while at Lake Janauarí, three copulations, two in early and one in late August (2002), were recorded. No egg chambers or offspring were found on tree trunks. The proportion of females with eggs was higher by the end of the aquatic phase in 2003 (Fig. 43). The rate of females with both mature and immature eggs increased significantly from August to September (mature eggs, $\chi^2 = 26.65$, $df = 1$, $P < 0.000$; immature eggs, $\chi^2 = 29.26$, $df = 1$, $P < 0.000$). The proportion of females without eggs was not significantly distinct comparing the two months. Nevertheless, females without eggs were observed only in August, whereas in September, close to the terrestrial phase, all dissected females carried eggs.

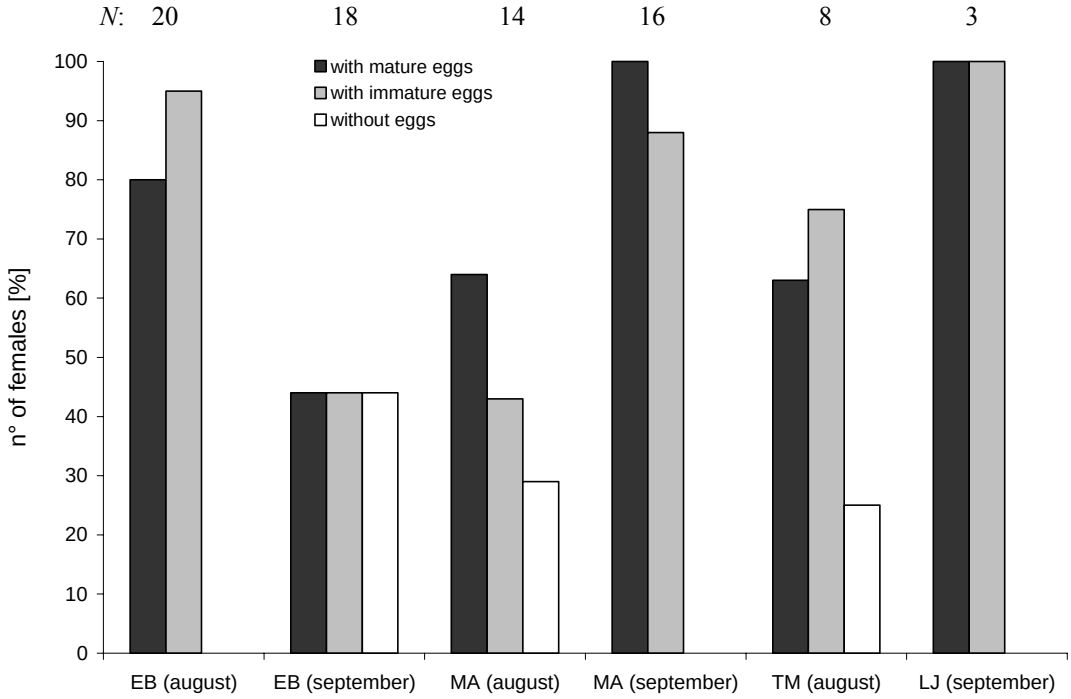

Figure 43. Proportion of females in *P. obliterata* with mature, immature and no eggs, collected in August and September 2003. Abbreviations: MA, Marchantaria Island; TM, Tarumã Mirím River; LJ, Lake Janauarí; and EB, CPPA Embrapa.

The average number of mature as well as immature eggs per female was also significantly higher towards the terrestrial phase (mature eggs, $P < 0.050$; immature eggs, $P < 0.010$; Kolmogorov-Smirnov test), with the quantity of mature eggs being higher than the number of immature eggs (Fig. 44). No significant difference was found for mature and immature eggs in monitored females from the different inundation forests (Kruskal-Wallis tests). When comparing MA and CPPA/Embrapa, significant differences in the number of mature eggs in September ($P = 0.000$) and in the quantity of immature eggs in both August ($P = 0.005$) and September ($P = 0.012$), were revealed. In August the number of immature eggs was higher in females from the plantation, while in September the number of both mature and immature eggs was higher in females from the inundation forest.

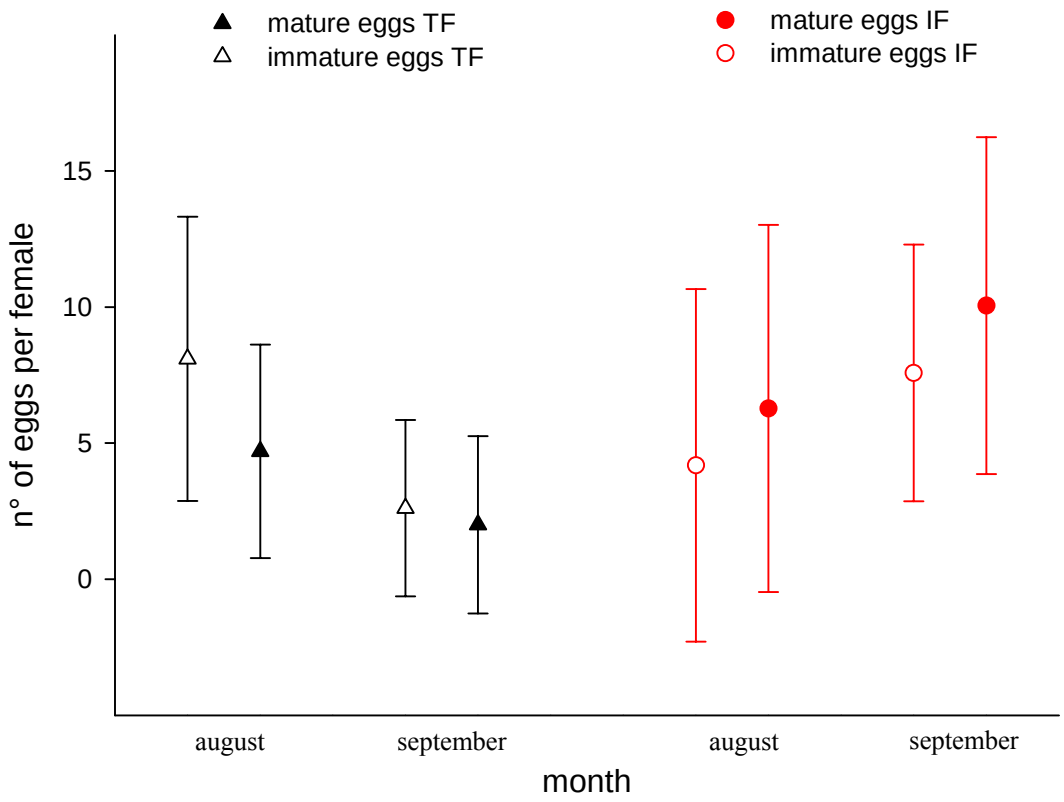

Fig. 44. Average number of mature and immature eggs in females of *P. obliterata* collected in August and September 2003. Abbreviations: TF, Terra firme (upland plantation), IF, inundation forests. Number of females dissected: TF, $N_{aug} = 20$, $N_{sep} = 18$; IF, $N_{aug} = 22$, $N_{sep} = 19$.

3.2.4.2.2 Banana Plantation

During the observation period, mating and continuous reproduction were recorded. The proportion of females carrying eggs varied in August and September 2003 (Fig. 43). Whereas all females yielded mature and/or immature eggs in August, the proportions of females with mature, immature and without eggs were equally distributed in September. The number of mature eggs per female was not significantly distinct between both months, but the quantity of immature eggs per female was significantly lower ($P < 0.025$; Kolmogorov-Smirnov-Test) in September than in August (Fig. 44).

3.2.5 Laboratory Experiments

3.2.5.1 Flooding Experiment

The migratory response of *P. obliterata* from seasonally flooded and upland areas to simulated successive flooding is shown in Figures 45 & 46. The two populations differed significantly in the maximum number of individuals per month on litter ($\chi^2 = 34.89$, $df = 4$, $P < 0.000$) and bark/container walls ($\chi^2 = 213.69$, $df = 4$, $P < 0.000$) in the course of the experiment. In both populations, millipedes only appeared on the litter surface after the irrigation treatment had started. For the inundation forest population from Marchantaria Island (MA), specimens on litter were relatively numerous in the beginning (n = 29, second month), with their numbers declining during the treatment (n = 3, fifth month; Fig. 45), due to the individuals taking refuge on bark/container walls. For the upland population from CPPA/Embrapa (EB), the number of individuals on litter was initially lower (n = 19), decreased further in the following month (n =1), but rose again during the last two months (n = 8, fifth month; Fig. 46). In both populations, the number of individuals located on bark/container walls increased after flooding had been initiated. The respective quantity was approximately twice as high for MA (n_{max}: 108, second month; Fig. 45) than for EB (n_{max}: 38, second month; Fig. 46), with both numbers declining during treatment, particularly on container walls, due to mortality apparently caused by limited resources and either a low or an excessive (condensed water at container walls) relative humidity. In both populations, a significantly higher number of millipedes was located on bark/container walls than on litter (MA: $\chi^2 = 200.22$, $df = 4$, $P < 0.000$, EB: $\chi^2 = 77.73$, $df = 4$, $P < 0.000$).

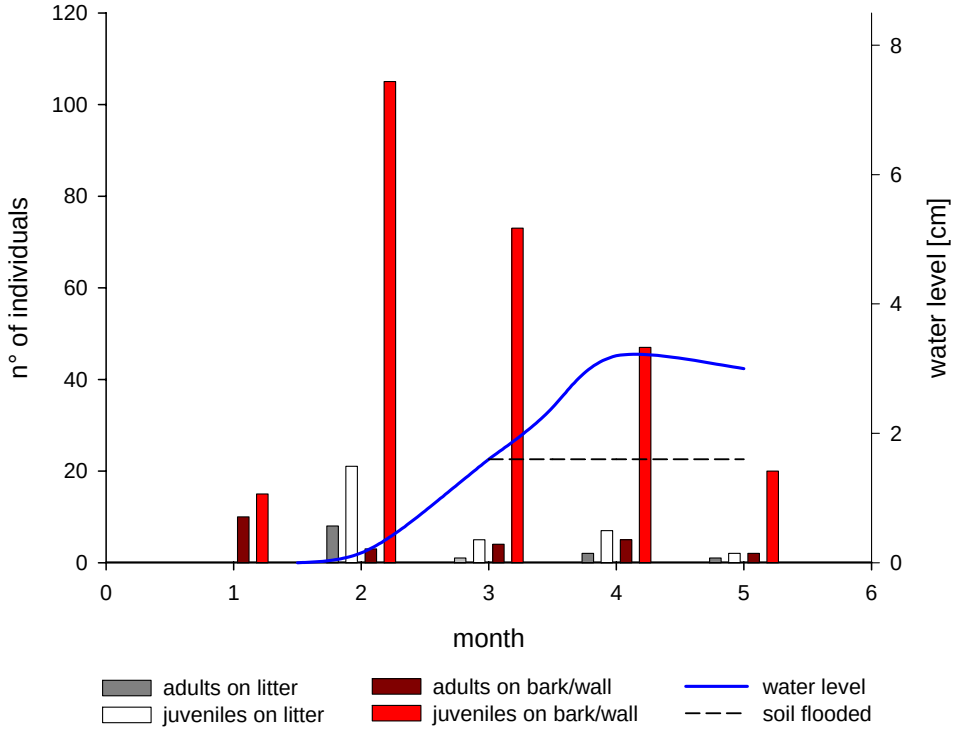

Figure 45. Response of *P. obliterata* from Marchantaria Island (whitewater inundation forest) to rising waters: vertical migration in an experimental flooding system. The columns show the maximum numbers of adults and juvenile stages that came to the litter surface and climbed bark/container walls per month. The blue line indicates successive flooding (months 2 to 4), the black marks the water level at which the soil was completely flooded. Number of specimens at onset of experiment (month 0): six adults (3 ♂, 3 ♀) and 26 juvenile stages.

The relative migratory response to flooding of both adults and juveniles observed on litter as well as bark/container walls did not differ significantly between populations ($Z < Z_{0.05(2)} = 1.960$). However, within both populations, the abundance of individuals on bark/container walls before and after flooding differed significantly for adults and juveniles (MA: $Z = 12.224$; EB: $Z = 6.556$; $P < 0.050$). Whereas the number of juveniles on bark/container walls increased from the first (control) to the second (treatment) month, the respective number of adults declined, apparently reflecting a general decrease in the number of adults due to natural death. Relative migration was significantly distinct for adults on bark/container walls and those on litter, since animals only appeared on the litter surface during treatment (MA: $Z = 16.282$; EB: $Z = 6.661$; $P < 0.050$). Differences are more pronounced for the MA population, as shown by higher Z values. In addition, a significant difference in the relative proportions of juveniles climbing bark and container walls was only observed for MA ($Z = 2.570$; $P < 0.050$). Whereas juveniles of both populations also occurred on bark during control, they only appeared on container walls after the irrigation treatment had started.

However, the number of juveniles taking refuge was higher for bark than for container walls (MA: 65/40, EB: 30/6 juveniles on bark/container walls, second month). Adults could not be found on container walls at any time, except for one individual of the MA population which was only found there on one occasion. Comparisons between the relative migration of adults and juveniles on litter as well as juveniles on litter and bark/container walls did not differ significantly within both populations. During control, sporadic mating and egg chambers on bark were recorded in both populations, but no such observations were made in the course of flooding. Only when the water level had fallen and the surviving millipedes returned to the ground reproduction was resumed.

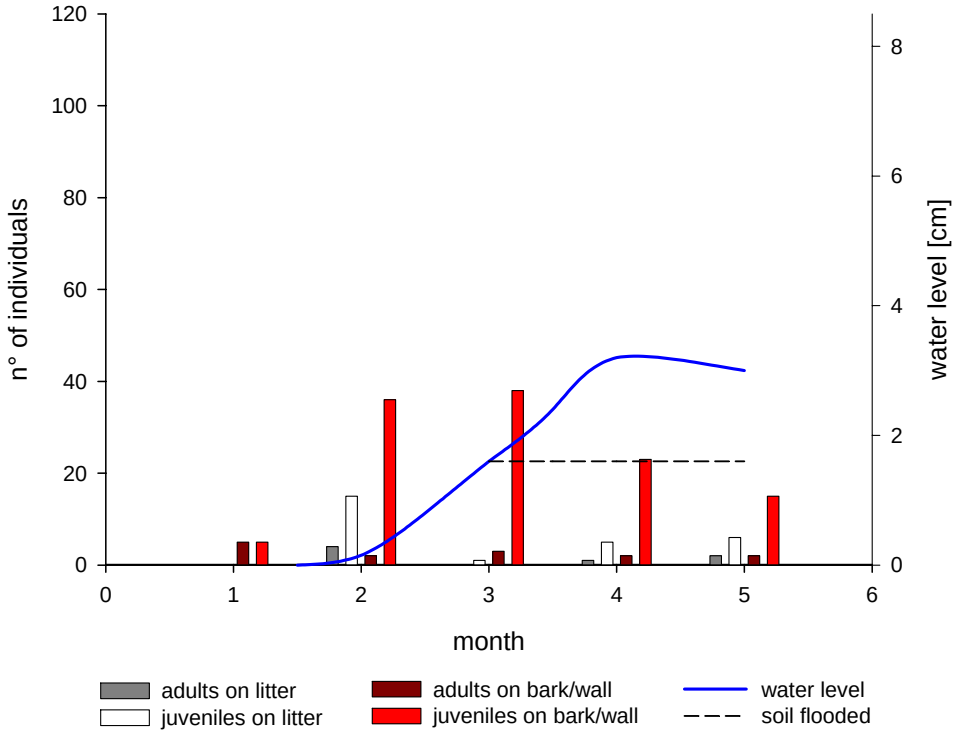

Figure 46. Response of *P. obliterata* from CPPA/Embrapa (upland banana plantation) to rising waters: vertical migration in an experimental flooding system. The columns show the maximum numbers of adults and juvenile stages that came to the litter surface and climbed bark/container walls per month. The blue line indicates successive flooding (months 2 to 4), the black marks the water level at which the soil was completely flooded. Number of specimens at onset of experiment (month 0): six adults (3 ♂, 3 ♀) and 26 juvenile stages.

3.2.5.2 Reproduction Experiment

P. obliterata deriving from both the upland plantation (EB) and the whitewater inundation forest (MA) reproduced in all conducted treatments with two different habitat substrates (i.e. earth and bark; Table 5). Egg chambers were first observed after five and six days in animals from the population EB and MA, respectively. Females

constructed egg chambers from all materials available, i.e. earth, plaster, bark and filter paper. The number of eggs per clutch (i.e. eggs in one chamber) ranged from five to 15 eggs in the population MA and from five to eleven eggs in the population EB. However, many vulnerable immatures died, mainly during ecdysis, the most sensitive period of development. The maximum number of clutches observed per pair of *P. obliterata* was four in MA and eight in EB, resulting in a maximum number of 28 and 55 first stage juveniles, respectively.

The linear mixed effect model showed significant effects of both treatment and habitat substrate on the development of resulting offspring during six months of observation (Table 11). Furthermore, the respective responses were significantly different for animals from the two populations (MA and EB). No significant interactions were revealed between treatment and habitat substrate, i.e. in each population the response to different treatments did not vary significantly with the applied habitat substrate and vice versa. Hence, to simplify comparative effects of both habitat substrate and treatment in the populations, approaches in which the other variable was held at a control state were considered as follows: the population-specific response to different treatments is regarded for treatments performed on earth, while the effect of different habitat substrates in the populations is examined referring to the control treatment. The oldest developmental stage occurring per month was used to demonstrate the developmental rate of offspring from both populations kept under different treatments and on different habitat substrates (Figs 47 & 48). Error bars are omitted in the respective figures, but descriptive statistical parameters such as standard deviations between replicates (pairs) are listed in Tables 12 & 13.

Table 11. Validation of effects and interaction terms (:) of fixed factors (treatment and habitat substrate, both nested within population) in a linear mixed effects model (dependent variable: oldest developmental stage; random factors: month and replicate pair, both nested within population). Numerator (numDF) and denominator (denDF) degrees of freedom as well as *F*- and *P*-values are listed. Bold numbers indicate significant effects accepting a 5 % significance level.

	numDF	denDF	*F*-value	*P*-value
(Intercept)	1	1654	107.355	<0.000
treatment	3	143	6.218	**0.001**
substrate	1	143	4.429	**0.037**
treatment:population	4	143	5.942	**0.000**
substrate:population	1	143	7.0152	**0.009**
treatment:substrate	3	143	0.471	0.703
treatment:substrate:population	3	143	1.593	0.196

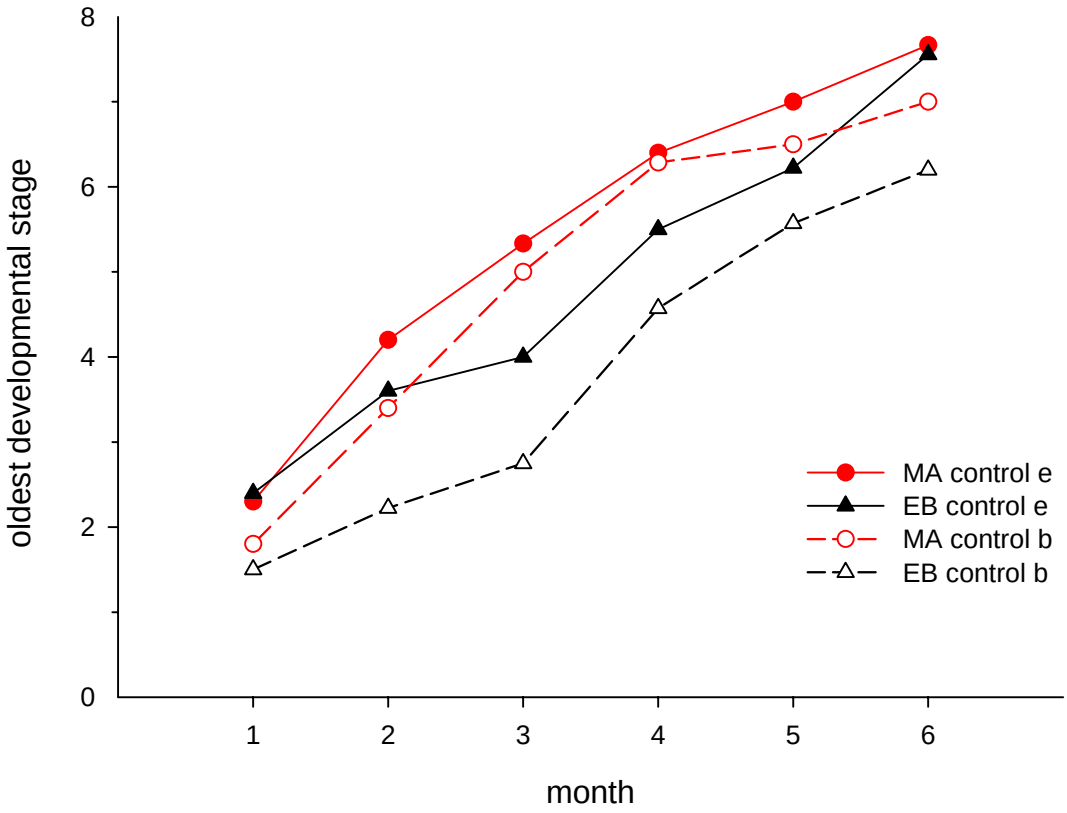

Figure 47. Developmental rate (average of oldest developmental stage per month) in offspring from pairs of *P. obliterata* from Marchantaria Island (MA) and CPPA/Embrapa (EB) cultured on earth (e) or bark (b). For descriptive statistics see Tables 12 & 13.

While all of the 80 pairs (replicates) kept on earth reproduced, only 7.5 % (six out of 80 pairs, two from EB, four from MA) of those maintained on bark did not. In terms of continuous reproduction, the population EB clearly surpassed the population MA in the length of the period in which new juveniles occurred (Table 14). When kept on earth, newly hatched offspring from EB could be observed during the entire observation period (six months), while animals from MA ceased to reproduce after the 3rd month at the latest. When kept on bark, pairs from EB only produced offspring up to the 4th month, whereas the habitat substrate had no influence on the reproductive behaviour of adults from MA.

In both populations, the development of the resulting offspring was generally faster on earth than on bark (Fig. 47; Table 12 & 13). Furthermore, immatures from pairs of the inundation forest population MA developed faster than those of the upland population EB. The MA offspring in the control treatment on earth revealed the most rapid maturation, with the first individuals turning adult in the 3rd month of observation.

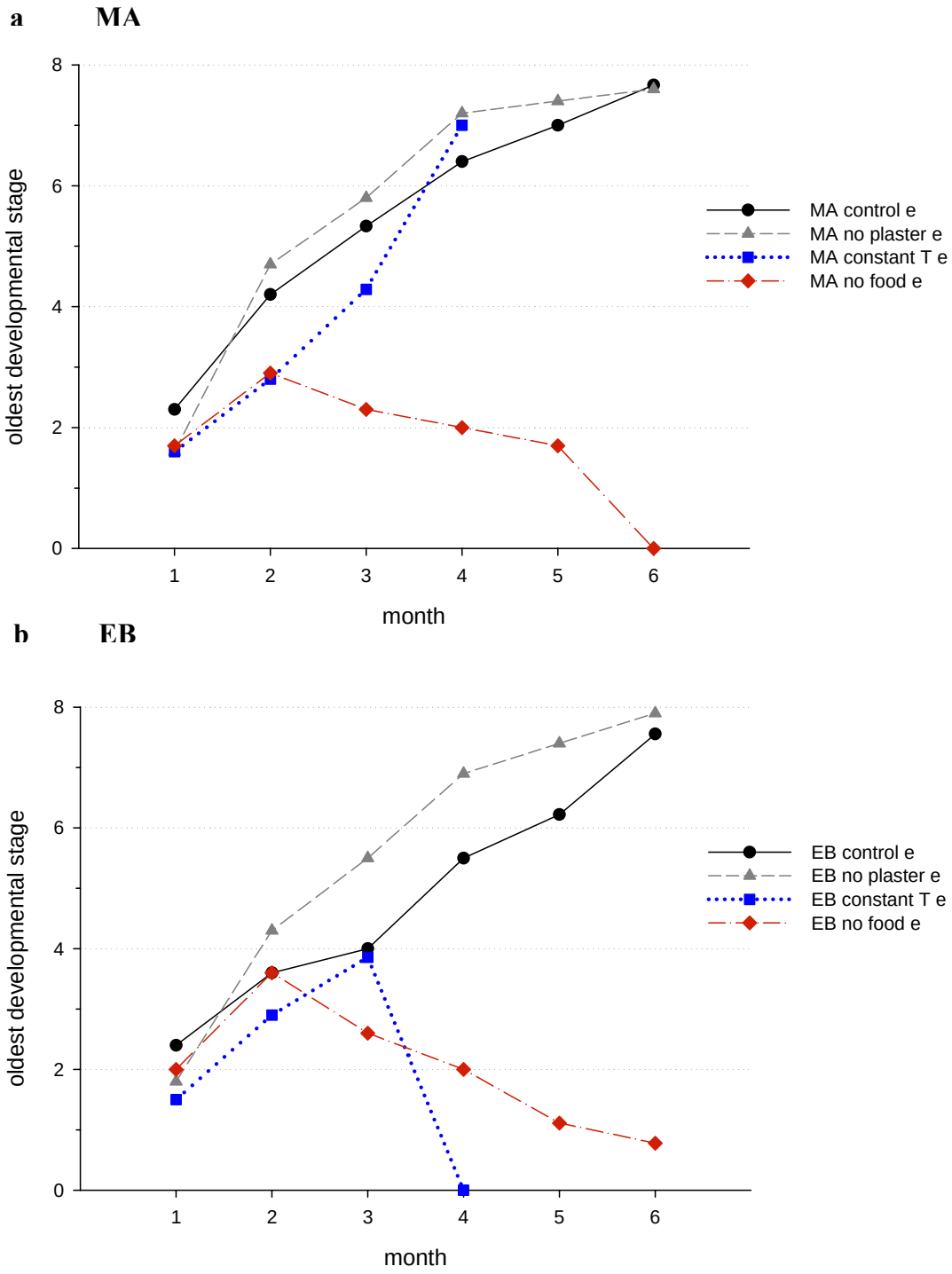

Figure 48a-b. Developmental rate (average of oldest developmental stage per month) in offspring from pairs of *P. obliterata* in four different treatments - control, no plaster, constant temperature (T), no food (Tab. 5) - cultured on earth (e). **a.** Marchantaria Island (MA). **b.** CPPA/Embrapa (EB). For descriptive statistics see Tables 12 & 13.

When kept on bark, all pairs reproduced, but the average development of immature stages was delayed for approximately 0.5 stages so that individuals first reached adulthood in the 5th month. Yet, the respective maturation and also the development rate during the 3rd to 5th months were comparatively faster than in the upland population EB.

Table 12. Statistical parameters of the oldest developmental stage in offspring from pairs of *P. obliterata* from Marchantaria Island kept in five different treatments (Tab. 5). The number of replicate pairs (*N*) and mean, standard deviation (SD) and minimum (X_{min}) and maximum values (X_{max}) for the oldest developmental stage per month are given. Abbreviations: MA, Marchantaria Island; e, earth; b, bark; and T, temperature.

	N	Mean	SD	X_{min}	X_{max}
MA control e					
month 1	10	2.3	0.9	1.0	3.0
month 2	10	4.2	0.6	3.0	5.0
month 3	9	5.3	1.4	4.0	8.0
month 4	5	6.4	0.5	6.0	8.0
month 5	5	7.0	0.7	6.0	8.0
month 6	3	7.7	0.6	7.0	8.0
MA control b					
month 1	10	1.8	0.8	1.0	3.0
month 2	10	3.4	0.8	2.0	4.0
month 3	10	5.0	0.7	4.0	6.0
month 4	7	6.3	0.5	6.0	7.0
month 5	6	6.5	1.4	5.0	8.0
month 6	3	7.0	1.0	6.0	8.0
MA no plaster e					
month 1	10	1.6	0.7	1.0	3.0
month 2	10	4.7	0.5	4.0	5.0
month 3	10	5.8	0.9	4.0	7.0
month 4	10	7.2	0.6	6.0	8.0
month 5	5	7.4	0.5	7.0	8.0
month 6	5	7.6	0.5	7.0	8.0
MA constant T e					
month 1	10	1.6	0.8	1.0	3.0
month 2	10	2.8	1.0	1.0	5.0
month 3	7	4.3	1.0	3.0	5.0
month 4	1	7.0		7.0	7.0
MA no food e					
month 1	10	1.7	0.8	1.0	3.0
month 2	10	2.9	1.4	0.0	5.0
month 3	10	2.3	2.1	0.0	5.0
month 4	10	2.0	2.6	0.0	6.0
month 5	10	1.7	2.8	0.0	7.0
month 6	10	0.0	0.0	0.0	0.0

On either habitat substrate, the EB offspring took no less than six month to reach the adult stage. Relative to MA, the average development of juvenile stages from EB kept on earth slowed down by almost one stage from the 2nd up to the 5th month of observation. When kept on bark, one replicate pair did not reproduce (compare 0 values

for X_{min} in Table 13), and the average development of the offspring slowed down by approximately one developmental stage.

Table 13. Statistical parameters of the oldest developmental stage in offspring from pairs of *P. obliterata* from CPPA/Embrapa kept in five different treatments (Tab. 5). The number of replicate pairs (*N*) and mean, standard deviation (SD) and minimum (X_{min}) and maximum values (X_{max}) for the oldest developmental stage per month are given. Abbreviations: EB, CPPA/Embrapa; e, earth; b, bark; and T, temperature.

	N	Mean	SD	X_{min}	X_{max}
EB control e					
month 1	10	2.4	0.8	1.0	3.0
month 2	10	3.6	0.7	2.0	4.0
month 3	10	4.0	0.7	3.0	5.0
month 4	10	5.5	0.7	4.0	6.0
month 5	9	6.2	0.4	6.0	7.0
month 6	9	7.6	0.5	7.0	8.0
EB control b					
month 1	10	1.5	1.1	0.0	3.0
month 2	9	2.2	1.3	0.0	4.0
month 3	8	2.8	1.9	0.0	5.0
month 4	7	4.6	2.2	0.0	6.0
month 5	7	5.6	2.5	0.0	7.0
month 6	5	6.2	3.5	0.0	8.0
EB no plaster e					
month 1	10	1.8	0.6	1.0	3.0
month 2	10	4.3	0.9	3.0	6.0
month 3	10	5.5	1.1	4.0	7.0
month 4	10	6.9	0.6	6.0	8.0
month 5	10	7.4	0.5	7.0	8.0
month 6	10	7.9	0.3	7.0	8.0
EB constant T e					
month 1	10	1.5	0.5	1.0	2.0
month 2	10	2.9	1.1	0.0	4.0
month 3	7	3.9	1.9	0.0	6.0
month 4	1	0.0		0.0	0.0
EB no food e					
month 1	10	2.0	0.5	1.0	3.0
month 2	10	3.6	0.5	3.0	4.0
month 3	10	2.6	1.9	0.0	5.0
month 4	9	2.0	2.4	0.0	5.0
month 5	9	1.1	2.2	0.0	5.0
month 6	9	0.8	2.3	0.0	7.0

Regarding the continuous reproduction on earth under different treatments, newly hatched offspring in the inundation population MA occurred up to the 4th month in the 'no plaster' treatment, i.e. one month longer than in the control (Table 14). The 'constant T' treatment had no effect on the reproductive output, while the breeding period was one month shorter in the 'no food' treatment. Pairs from the upland population EB reproduced during the whole observation period of six months in both control and 'no plaster' treatment. The relative effect of 'constant T' and 'no food' was more severe for EB than MA, since the breeding period for adults from EB was reduced to predominantly two months in both treatments.

Table 14. Period in which new offspring was produced by *P. obliterata* from floodplains (Marchantaria Island) and uplands (CPPA/Embrapa) in eight different rearing treatments (Tab. 5). Data give the duration of hatching of first juvenile stages per treatment during six month observation period. Abbreviations: MA, Marchantaria Island; EB, CPPA/Embrapa; e, earth; b, bark; and T, temperature.

Treatment	Offspring **MA**	**EB**
control e	3 months	6 months
control b	3 months	4 months
no plaster e	4 months	6 months
no plaster b	3 months	5 months
constant T e	3 months	2 months
constant T b	2 months	3 months
no food e	2 months	2 months
no food b	2 months	2 months

For the inundation forest population MA, the control and 'no plaster' treatments yielded the fastest development of offspring (Fig. 48a; Table 12). From the 2nd to the 5th month, juveniles were on average approximately 0.5 stages older in the 'no plaster' treatment than in the control. The first adults, however, were observed in the 3rd month of the control, but only in the 4th month of the 'no plaster' treatment. Not all of the hatched individuals reached maturity during the six months of observation. In the 'constant T' treatment, the average development in offspring was slowed down by more than one stage during the 2nd and 3rd months compared to both former treatments. The oldest immature stages encountered during the four months of observation were subadult. The effect of food deprivation on offspring development was even more severe, since all immatures died during the 'no food' treatment, with the oldest developmental stages being subadult in the 5th month.

In contrast, for the upland population, the fastest development in all months except the first one was observed in the 'no plaster' treatment (Fig. 48b; Table 13). The resulting offspring were on average more than one stage older than in the control, and the first individuals reached adulthood in the 4th month compared to the 6th month in the control. Not all of the hatched millipedes accomplished maturation during the six months of observation. In the 'constant T' treatment, the immatures of one replicate pair died during the four months of observation (compare 0 values for X_{min} in Table 13). On average, the development of offspring during the first three months slowed down compared to the 'no plaster' treatment, but was similar to that of the control. The oldest immature stages encountered during three months of observation reached the 6th developmental stage. In the 'no food' treatment, most immatures died, but those of one replicate survived until the 6th month and attained the subadult stage.

The mortality rate of maturing individuals prior to the reproduction experiment differed significantly among populations, but neither between habitat substrates nor between the control and 'constant T' treatments (Fig. 49a; Table 15). Mortality was significantly higher for individuals from the population MA (on average: 34 %) than for EB (on average: 10 %).

Mortality in adults during the reproduction experiment was significantly distinct, i.e. higher on bark compared to earth as well as in the treatments 'no plaster' and 'no food' compared to the control (Fig. 49b; Table 16). Both the population and 'constant T' treatment had no significant impact on the mortality of adults. As shown by the low odd ratios, the relative deleterious effect of the variables on the survival of adults was most pronounced for the 'no food' treatment, i.e. food deprivation (average mortality: 90 %), followed by the 'no plaster' treatment and finally the bark as habitat substrate. Both 'no plaster' treatment and bark substrate were only notably lethal in their combination (average mortality: 61 %), in creating markedly dry conditions. Due to the high mortality rate, more individuals had to be replaced in the 'no plaster' and 'no food' treatments, resulting in higher *N* values.

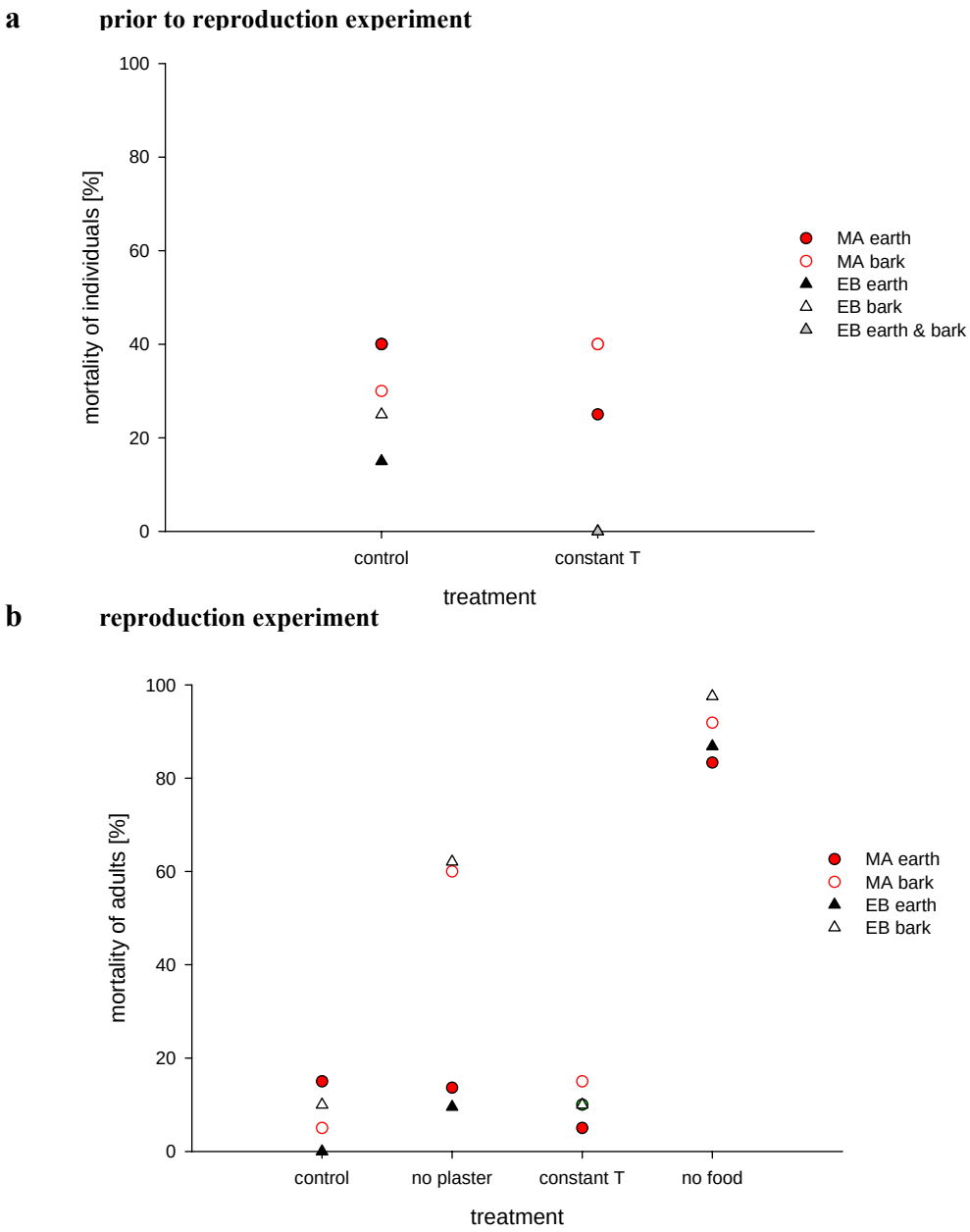

Figure 49a-b. Mortality in *P. obliterata* from Marchantaria Island (MA) and CPPA/Embrapa (EB) in four different treatments - control, no plaster, constant temperature (T), no food (Tab. 5) - cultured on earth and bark, respectively. **a.** Prior to reproduction experiment (6th juvenile stage and subadults). **b.** Reproduction experiment (adults). Number of individuals per treatment as indicated by indices ($N_{control}$-$N_{no\ food}$): MA, on earth: $N_{control} = 20$; $N_{no\ plaster} = 22$; $N_{constant\ T} = 20$; $N_{no\ food} = 36$. MA, on bark: $N_{control} = 20$; $N_{no\ plaster} = 40$; $N_{constant\ T} = 20$; $N_{no\ food} = 37$. EB, on earth: $N_{control} = 20$; $N_{no\ plaster} = 21$; $N_{constant\ T} = 20$; $N_{no\ food} = 38$. EB, on bark: $N_{control} = 20$; $N_{no\ plaster} = 29$; $N_{constant\ T} = 20$; $N_{no\ food} = 41$.

Table 15. Effect of treatment, habitat substrate and population on the mortality of maturing individuals of *P. obliterata* kept prior to reproduction experiments. Impacts of variables were evaluated by a multinominal logistic regression, using control (treatment), earth (substrate) and EB (population) as a reference group. The effect coefficient (*b*), standard error (SE), degrees of freedom (*df*), significance (*P*) and odds ratio (exp (*b*)) are given. Abbreviations: EB, Embrapa; MA, Marchantaria; and T, temperature.

	b	SE	*df*	*P*-value	exp (*b*)
(Intercept)	2.012	0.459	1	0.000	
treatment (constant T)	0.734	0.412	1	0.075	2.083
substrate (bark)	-0.246	0.406	1	0.545	0.782
population (MA)	-1.558	0.447	1	0.000	0.211

Table 16. Effect of treatment, habitat substrate and population on the mortality of adult *P. obliterata* reared in reproduction experiments. Impacts of variables were evaluated by a multinominal logistic regression, using control (treatment), earth (substrate) and EB (population) as a reference group. The effect coefficient (*b*), standard error (SE), degrees of freedom (*df*), significance (*P*) and odds ratio (exp (*b*)) are given. Abbreviations: EB, Embrapa; MA, Marchantaria; and T, temperature.

	b	SE	*df*	*P*-value	exp (*b*)
(Intercept)	3.487	0.519	1	0.000	
treatment					
(no plaster)	-2.186	0.478	1	0.000	0.112
(constant T)	-0.326	0.575	1	0.570	0.721
(no food)	-5.158	0.544	1	0.000	0.006
substrate (bark)	-1.517	0.325	1	0.000	0.219
population (MA)	0.030	0.285	1	0.916	1.030

3.3 Discussion

3.3.1 Abundance in Different Biotopes

Studies across a wide variety of organisms have demonstrated that widespread species also tend to be locally abundant (Brown 1984; Gaston 1996; Gaston 1996). Symonds & Johnson (2006) have further suggested that such positive range size-abundance relationships may rather apply to recently evolved or recently expanding species, the latter assumed to be the case in *P. obliterata* (Chapter 4.3.3). However, the correlation between range size and local population densities is not always purely linear. Some authors, e.g. Brown & Maurer (1987) and Gaston (1994), described cases, where species with wide distributions vary from being locally abundant to locally rare.

Millipede communities have been stated earlier to differ between biotopes in the density of individual species (Bhakat 1989). Accordingly, I found the occurrence of *P. obliterata* to vary among the studied Central Amazonian biotope types. These included two non-flooded upland (Terra firme) locations: the banana plantation at CPPA/Embrapa (EB), and the primary forest at 'Reserva A. Ducke' (RD); as well as three different seasonal inundation forest sites: Marchantaria Island (MA: whitewater, i.e. Várzea), Tarumã Mirím River (TM: blackwater, i.e. Igapó), and Lake Janauarí (LJ: mixedwater, i.e. Várzea & Igapó).

While *P. obliterata* occurred most frequently, and was the dominant millipede species, both on the plantation at EB and in the whitewater inundation forest on MA, it was less common at the studied black- and mixedwater inundation forest sites, where coexisting millipede species were more abundant (Tables 8 & 10). Furthermore, the species *P. obliterata* was not encountered in the primary forest at RD which only supported few foreign specimens (Chapter 3.1.1.4.2).

As with other species, local variations in the relative abundance of *P. obliterata* can probably be accounted for by the species' colonisation history, as well as by regional abiotic conditions and species interactions. The observed biotope-specific abundances are thus discussed here with respect to the species' modes of distribution, and both the availability of, and the competition for, local resources.

The species *P. obliterata* is believed to have originated from the Andean foreland forests in Peru (Golovatch & Sierwald 2001) and seems to represent an invasive form relatively new to the study area (Chapter 4.3.3). The dispersal capacity of a small millipede is naturally limited (Hopkin & Read 1992), however the species' immigration

is most likely promoted by passive distribution along the rivers on the one hand, and, since specimens on plantations are believed to have arrived from imported palm seeds, introductions with plant material by man on the other hand (Chapters 4.3.1.1 & 4.3.1.3.3). Therefore, in the Brazilian region of Central Amazonia, the millipede *P. obliterata* is more likely to colonise seasonally inundated forests along the rivers and anthropogenic plantations rather than upland forests that are more difficult to access.

The downstream dispersal of migrants from source populations of *P. obliterata* in Peru is restricted to biotopes along the Solimões/Amazon River, i.e. mainly to Várzea and also mixedwater locations (Fig. 2). Since the inundation forest on MA is isolated from the mainland, its invasion by non-flying, terrestrial invertebrates is restricted to introductions via waterways. This evidently accounts for the local dominance of the thus dispersed *P. obliterata*, and also for the presence of its less common congener *P. insularis* (Bergholz et al. 2005).

Sediment analyses at the northern banks of the Negro River upstream of Manaus (i.e. the confluence of the two rivers; Fig. 2) indicate an occasional inflow of waters from the Solimões River (Furch 1999; Irion, pers. commun.), and this is believed to account for the occurrence of *P. obliterata* at the studied Igapó sites at the lower Negro River (Chapter 4.3.1.3.3). Gene flow from the source populations in Peru is nevertheless mostly confined to subpopulations along the Solimões/Amazon River, with the subpopulations in blackwater forests upstream of Manaus, such as at TM, receiving less replenishment. Therefore, the Igapó sites are expected to harbour comparatively lower population densities of *P. obliterata* than the Várzea locations.

Hence, with respect to colonisation history, as well as current introductions, both the Várzea habitats and the plantation sites are expected to comprise higher frequencies of *P. obliterata*. These results furthermore correspond to the theory that Várzea floodplains adjacent to rivers, which are characterised by permanent relocation of sediments (Sioli 1984a), are supposed to be inhabited mainly by pioneer fauna, i.e. opportunists and generalists with great mobility (Adis 1997) like *P. obliterata* (Chapter 4.3.1.4).

However, in addition to the above described introductions via waterways, former secondary immigrations of terrestrial invertebrates from Terra firme into adjacent seasonally flooded biotopes are postulated for Igapó areas (Adis 1997). Most likely, such invasions have also occurred in Várzea or mixedwater locations that are connected to upland areas. As a result, these locations were probably previously inhabited by other small millipedes (Chapters 3.2.2.2 & 3.2.3.1.1) such as *Cutervodesmus adisi*, the

dominant species at the studied Igapó site at TM (Adis et al. 1996a), and *Docodesmus amazonicus*, the most abundant species in the mixedwater forest at LJ (pers. obs.). The presence of these species might have negatively influenced the local establishment of *P. obliterata* individuals that were subsequently washed ashore. As described for other millipede species in biotopes of the seasonal tropics, where the diversity of available food resources is low, the first species to colonise is likely to leave only limited resources for additional species (Dangerfield & Telford 1992), thus complicating the settlement of successive ones. As a consequence, abundances of *P. obliterata* are expected to be lower in areas occupied by coexisting species which possibly compete for the same resources.

In the agricultural systems at EB, *P. obliterata* is also not the only resident Diplopoda species. At least six other allochthonous millipedes have been recorded in the domain, apparently all of them introduced by man (Höfer et al. 2001; Bergholz et al. 2004). However, on the studied plantation site, *P. obliterata* outnumbered the only co-occurring small millipede species, *Myrmecodesmus hastatus*, by far (Table 10).

As stated above, competitive effects become most apparent in biotopes with restricted resources. While the plantation at EB is fertilised (Chapter 3.1.1.4.1), and the Várzea is rich in bioelements (e.g. calcium and phosphorous) due to sediment load carried by the whitewater river, the soils of both Igapó and Terra firme are comparatively poor in nutrients (Furch 1997; Vohland et al. 2003). The conditions in the nutrient-rich plantation and Várzea sites (Parolin et al. 1998) are more favourable for plant growth and probably also for decomposers such as *P. obliterata*, particularly since Neotropical millipedes are assumed to have originated from the bioelement-rich Andean region (Vohland et al. 2003).

Besides lower immigration rates via waterways, enhanced competition with established millipede species for the locally restricted resources could contribute to the lower population densities of *P. obliterata* at the studied Igapó and mixedwater sites. This seems to be reflected by the fact that the congener *P. insularis*, which is believed to share both origin and dispersal by the Solimões/Amazon River with *P. obliterata* (Kraus 1960; Golovatch & Sierwald 2001; Bergholz et al. 2005), is absent from the studied Igapó at TM. Since *P. insularis* is generally low in abundance, it is apparently a poor competitor and has not succeeded to colonise the blackwater location, whereas repeated introductions by the river may help to sustain the small local populations at the Várzea and mixedwater sites. A superior competitor and coloniser, *P. obliterata* also invaded

the blackwater location although it shows the highest population density on MA. Here, in addition to the constant population replenishment by migrants from the area of origin in Peru, conditions are favourable due to higher resources on the one hand, and very low numbers of co-existing foreign millipede specimens on the other hand.

The *P. obliterata* population on the nutrient-rich plantation at EB apparently also benefited from low competition and high-quality food. Such optimal local conditions could explain why the millipede *P. obliterata* evidently refuses to invade and colonise neighbouring, bioelement-poor upland forests in substantial densities. Five other anthropogenically introduced millipede species were also especially numerous outside the primary forest, i.e. in agricultural systems (Höfer et al. 2001). At first this seems contradictory to the observation that both air and soil temperatures are higher at these sites than in the nearby primary and secondary forest, where a well-developed canopy protects macrofauna from high temperature variation and drought stress (Martius et al. 2004). Despite the less favourable climatic conditions, Diplopoda, and in particular the pyrgodesmids, nevertheless dominated the decomposer guild (7 to 9 % of all arthropods) on local plantations (Höfer et al. 2001). However, tropical forest floors are generally characterised by scarcity of humus (Prinzing & Woas 2003), while high-quality litter is often decomposed very rapidly, with the available litter for detrivores often containing phenols and tannins (Dangerfield 1993). Litter with high concentrations of these compounds is not easily digested by detrivores. A predominance of polydesmids on fields compared to forests was also observed in a long-term study of European millipede communities (Tajovsky 1993). Tajovsky (1993) suggested that polydesmids may be better r-strategists than other millipede colonisers, and that food resources belong to the factors determining the distribution of millipedes. Such an influence of food quality on local abundance has already been observed for the Central Amazonian *Pycnotropis* species which inhabit both the Várzea (*P. tida*) and non-flooded areas (*P. sigma*) (Vohland et al. 2003). According to Vohland et al. (2003), *P. sigma* was abundant on a manured plantation, whereas its population densities in the surrounding upland forests were so low that hardly any specimens were found. This corresponds to the observed local abundances of *P. obliterata* on the plantation at EB and in the primary forest at RD.

The importance of food quality would also explain why *P. obliterata* abandoned the palm plantation that it inhabited before (Adis et al. 2001), in favour of the banana plantation. The latter plantation type also attracts other tropical polydesmids, e.g.

M. hastatus (Bergholz et al. 2004) and *Cylindrodesmus hirsutus* (Bergholz 2006). Ashwini & Sridhar (2005) showed that among five diets assessed, the tropical millipede *Arthrosphaera magna* revealed a secondary preference for the consumption of banana litter. The same favouritism may apply to *P. obliterata*, and could be due to the high water content of the plants (Chapter 3.2.1.4), since the acceptance of decomposing material by millipedes correlates with moisture (Sakwa 1974).While the animals did not accept fresh plant material, moist pseudo-stems in an advanced state of decay were, in part, densely colonised by *P. obliterata* (Chapter 3.2.3.2). Such stems probably support the growth of fungal mycelia and algae that, according to Hassal et al. (1986), become more palatable or easier to digest for arthropods when moistened. The moisture may also activate microbiota that act to release available food substances from complexes and increase the palatability of the banana litter (Sakwa 1974). Hence, the decomposing pseudo-stems then provide access both to food, and to sufficient moisture for the animals. The influence of these two factors in the selection of favourable tree stumps has already been reported for a European millipede, *Proteroilius fuscus* (Peitsalmi 1981).

3.3.2 Life History Traits

3.3.2.1 Seasonal Vertical Migration

Terrestrial invertebrates in Central Amazonian inundation forests are divided into two groups; (1) terricolous, i.e. ground-dwelling, and (2) arboricolous, i.e. tree-living animals (Adis 1992b). Both groups include 'migrants' and enduring 'non-migrants'. The latter complete their life cycles in only one habitat and are either active underwater or remain dormant during flooding. In contrast, the migrants change habitats during their life cycles to escape the seasonal inundation, although they reproduce primarily in one habitat (Adis 1997). According to Adis (1997), three modes of temporary migration are distinguished among the terricolous migrants; firstly, horizontal migration following the water line; secondly, vertical migration to trunk or canopy; and thirdly, flight to non-inundated upland areas.

It is not surprising that among the non-flying, resident terrestrial arthropods, particularly millipedes tend to escape rather than trying to outlast inundation. Millipedes are well-known for their 'wandering tendency' (Cloudsley-Thompson 1949), and are thus likely to adapt to severe conditions by means of migratory movements. The main evolutionary

advantage of migration should involve enabling a species to colonise changing or temporary habitats (Southwood 1962). Since the theory of optimal habitat selection predicts that individuals will move among habitats to maximise their fitness (Holt 1985), migration tendency is maximal in the most impermanent biotopes (Southwood 1962) such as Central Amazonian floodplains. Due to their limited dispersal rate (Hopkin & Read 1992), the small and slow millipedes naturally rather climb nearby trees, i.e. perform vertical migrations, than move horizontally like other resident invertebrates that migrate to adjacent upper, non-flooded forest areas (cf. Irmler 1979).

Concordantly, seasonal vertical migration, with an arboreal mode of life exclusive to the aquatic phase, has been reported for most resident millipedes, i.e. *Cutervodesmus adisi* (Adis et al. 1996a), *Pycnotropis tida* (Vohland & Adis 1999), *Mestosoma hylaeicum* (Adis 1992a), *Prostemmiulus adisi* (Mauriès 1984), as well as *Poratia insularis* and *Docodesmus amazonicus* (pers. obs.). The same has been assumed for *P. obliterata* since the species was found on tree trunks during flooding (Messner & Adis 1988). Due to its small body and paraterga as well as relatively strong legs, *P. obliterata* appears to be a good climber, as has been suggested for *C. adisi* (Adis et al. 1996a).

Alternative, non-migratory survival strategies have, so far, only been shown for two millipede species. One of them is the strictly arboricole *Epinannolene arborea* (Adis 1984) that permanently inhabits tree trunk and canopy regions. However, my field observations confirmed that such a permanent arboreal mode of life does not apply to *P. obliterata*; as soon as the water retreated from the forest floor, the species returned to the ground (Chapter 3.2.3.1.1). Before and after flooding, i.e. during the terrestrial phase, the millipedes dwelled in the moist forest litter and topsoil, and occasionally also inhabited fallen dead wood. They were, however, never observed to climb, or reside on, living trees. Similarly, animals on the non-flooded plantation site ascended neither banana plants nor sporadic trees, but dwelled in the moist pseudo-trunks lying on the ground (Chapter 3.2.3.2).

The same terricolous behaviour was observed for specimens kept in the laboratory (Chapter 3.2.5.1). Before experimental flooding, the millipedes, from both seasonally inundated and upland sites, treated bark pieces as dead wood. Only a few individuals, in particular those from the inundation forest population, colonised the bark. However, when their artificial habitat was successively flooded, the soil-dwelling individuals of both populations escaped to upright areas. Thus, the ability for vertical migration to avoid drowning is present in *P. obliterata* from both seasonally inundated (MA; Fig.

45), and non-flooded plantation sites (EB; Fig. 46). In the field I also observed that specimens of both biotope types responded to dry conditions by retreating to moister microhabitats (Chapter 3.3.2.2.1). Hence, in all biotopes, the species *P. obliterata* reacts to acute excessive or insufficient water saturation in its surroundings. This suggests that the species might be polymorphic with respect to migratory movements as defined by Southwood (1962). Field and experimental observations further supported this theory by showing all developmental stages of *P. obliterata* to exhibit trunk ascent (Chapters 3.2.3.1.1 & 3.2.5.1), whereas in most other resident terrestrial arthropod species only definite life stages accomplish the seasonal vertical migration (Adis 1981, 1992b). However, during the aquatic phase the advanced stages (juveniles of the 6th stage and subadults; Chapter 3.3.2.3.1) definitely outnumber other stages and can thus be referred to as preferential migratory stages of *P. obliterata*. Although the two different populations showed a similar response to rising waters in the flooding experiment, the number of specimens responding was twice as large for the inundation forest population than for the upland plantation one (Figs 45 & 46). Since pre-treatment reproductive outputs are assumed to be higher for the upland population (cf. Chapter 3.3.2.3.3), this suggests a higher migration readiness for *P. obliterata* from seasonally flooded locations. The local specimens have probably evolved a predisposition for such evasive movements, i.e. an ethological adaptation to the annually flooded biotopes.

Besides the above-mentioned arboricole *E. arborea*, the single other non-migratory millipede species in the region is *Myrmecodesmus adisi* (Messner & Adis 1988). *M. adisi* is the only native millipede that shows a submersion resistance for up to eleven months, accomplished by means of plastron respiration (Adis & Messner 1997). This adaptation is confined to its semi-aquatic advanced immature stages, which pass inundation under loose bark on submerged tree trunks. In fact, *P. obliterata* and some other polydesmids (e.g. *P. tida* and *M. hylaeicum*) show submersion tolerances that range from a few hours or days up to several weeks (Adis & Messner 1997). *P. obliterata* individuals of seasonally flooded forests are capable of surviving submersion for up to 68 hours and are thus more tolerant to inundation than specimens from non-flooded sites (Wilck 2000). Still, due to an incomplete or absent plastron, long-term inundation is fatal to all local millipedes except *M. adisi* and thus the litter-dwelling animals must evade flooding to survive.

For the migrants in the resident terrestrial arthropod fauna, migration is facultative and mainly triggered off by some factor that heralds the advance of inundation (Southwood

1962; Adis et al. 1997). Whereas the flood pulse is the original cause for up- and downward movements, it remains the primary control mechanism only among certain species (Adis 1992b). Most invertebrates, like the millipede *C. adisi*, have become sensitive to secondary, mainly abiotic factors (Adis et al. 1996a). Their migration is now triggered predominantly by the rainy season (December to May), beginning three to four months before flooding, and the resulting changes in edaphic and climatic factors, e.g. increasing wetness of the soil, the relative air humidity, and decreasing differences between the maximum and minimum temperature near the forest floor (Adis 1997). In *C. adisi*, trunk ascent thus begins several weeks before the forest inundation (Adis et al. 1996a). In contrast, I found that, in the inundation forests, seasonal migrations of *P. obliterata* occur in direct response to the advancing or receding waters (Chapter 3.2.3.1.1). As with specimens in the laboratory experiment (Chapter 3.2.5.1), *P. obliterata* escape flooding only shortly before the actual inundation of their habitat, just like the local Opiliones, Isopoda and some species of Aranae do, whose trunk ascents are not correlated with climatic factors (Adis 1997). Given that the species *P. obliterata* is still relatively new to the area (Chapter 4.3.3), it has possibly not yet had sufficient time to evolve an additional sensitivity to preceding climatic changes, and thus still responds primarily to the advancing waters.

Vertical migrations of the local arthropods are also influenced by macroclimatic El Niño-Southern Oscillation (ENSO) events (Adis & Latif 1996). According to Adis and Latif (1996), in years without El Niño, trunk ascents of most invertebrates were mainly correlated with changes in the local climatic conditions. In a strong El Niño year, however, they found no such correlations. Instead, due to the lower precipitation in Central Amazonia and the thus low-water discharge of the Amazon River, both rainy season and forest flooding were delayed and the lagged trunk ascent of all arthropods was related to the onset of inundation (Adis & Latif 1996). This shows flexibility in the species now primary responding to secondary factors since, in the absence of such preceding stimuli, the animals subsequently responded to the original factor, i.e. forest flooding. However, the animals appeared to suffer a decrease in population densities due to the extreme abiotic situation (e.g. relative dryness), and this was only recovered in the following years (Adis & Latif 1996). Such population fluctuations, due to macroclimatic changes, also seem to occur in *P. obliterata*. The year 2003 was characterised by a moderate El Niño, i.e. forest flooding was delayed for one month (Figs 17 & 18) and the precipitation pattern was different (Fig. 16b) compared to the

previous, more normal year 2002. As a consequence, fewer individuals were encountered on tree trunks in 2003 than in 2002, particularly in the black- and mixedwater inundation forests, where the species is less common (Table 8). In *P. obliterata*, the respective lower densities can probably be ascribed to a modified age structure and thus mortality in the local populations (Chapter 3.2.2.3.1). Due to delayed trunk ascent, the previous reproductive phase has apparently been extended, resulting in a larger fraction of juveniles that are even more dependent on moisture and thus susceptible to desiccation. In contrast, the upland population from the banana plantation seemed unaffected by the more extreme, drier climate, probably due to still sufficient humidity within the rotten plant material. Here, the total number of individuals encountered during observations differed only slightly between 2002 and 2003 (Table 10).

Despite the high overall tree diversity (Ferreira et al. 2005), *P. obliterata* were only found on a total of ten different tree species (and sporadically on trees which could not be specifically identified). Even among this limited range, *P. obliterata* showed a clear preference for one tree species, namely *Macrolobium acaciifolium* (Chapter 3.2.3.1.1). Although the millipedes colonised at least four tree species at each of the three different inundation forest sites, the majority of individuals were always encountered on *M. acaciifolium*. This favouritism may be attributed to the species' requirements with respect to bark microhabitats (Chapter 3.3.2.2.1). The trees of choice were those that generally consisted of coarse and loose-fitting bark. As the bark of many tropical forest species is very smooth (Vareschi 1980), providing few crevices and shelter, this could be one of the reasons why I found *P. obliterata* on only a relatively small number of tree species. To some extent, such a qualitative selection of the temporary refuge became apparent in my flooding experiment, where the animals preferred to climb rough bark rather than smooth container walls (Chapter 3.2.5.1). This behaviour was conspicuous in experienced adults (collected from tree trunks beforehand) that completely avoided container walls and chose only the more adequate bark substrates to pass the experimental inundation. Tree preferences have not yet been reported for other local millipede migrants, but it has been suggested that *C. adisi* might also avoid tree trunks with smooth bark (Adis et al. 1996a). Since the respective requirements for *C. adisi* and *P. obliterata* are similar, the observed preference for trees with rather coarse and loose-fitting bark is most likely not a species-specific attribute but rather a general phenomenon in the resident millipedes.

3.3.2.2 Microhabitat Selection and Social Behaviour

3.3.2.2.1 Choice of Adequate Microhabitats

Earlier studies showed that usually field millipedes are quiescent in the daytime and active during night hours (Peitsalmi 1981; Hopkin & Read 1992; Vohland & Adis 1999; Hamazaki 1996). This corresponds to my observation that, along with other Diplopoda residing on tree trunks (e.g. *Poratia insularis*, *Cutervodesmus adisi* and *Docodesmus amazonicus*) *P. obliterata* seem to rest during the day throughout the aquatic phase in seasonally inundated forests. Being nocturnal, the millipedes most likely disperse at night to forage for soft, soaked plant or bark material that is probably more palatable due to its moisture content (Chapter 3.3.1), as observed for the resident *Pycnotropis tida* (Vohland & Adis 1999). Algae may represent a considerable fraction of the local diet, given that some other tropical millipedes living on tree trunks were found to feed exclusively on the algae growing on bark (Mahsberg 1996).

During the day the heliophobe species *P. obliterata* seeks refuge under loose bark, apparently to protect itself from both insolation, and predatory arthropods (e.g. Chilopoda and Aranae) which also temporarily colonise the non-inundated trunk region (Chapter 3.2.3.1.1). Such a self-concealing behaviour has likewise been reported for other resident millipedes, including *C. adisi* (Adis et al. 1996a), *Myrmecodesmus adisi* (Adis & Messner 1997), *Epinannolene arborea* (Adis 1984) as well as *P. insularis* and *D. amazonicus* (pers. obs.). Due to its inconspicuous coloration and small size, *P. obliterata* are generally well-disguised. When their bark cover was removed during sampling the millipedes tended to either adhere motionless to the surface, or rapidly escape under adjacent bark pieces. It is likely that they show a similar reaction in the presence of predators. Most of the resident predatory arthropods are comparatively large, and hence lingered under bark pieces providing more space than those frequented by *P. obliterata*. The only predators able to invade its bark shelters appear to be small ants. Given that individuals of *P. obliterata* were occasionally found unharmed in the company of ant specimens, there may however be no interference at moderate ant densities and if easier prey is available. Like other millipedes (Hopkin & Read 1992), advanced developmental stages of *P. obliterata* feature a solid, calcified tegument and, when irritated, the animals secrete repelling liquid, i.e. hydrocyanic acid, from their defensive glands (Schubart 1955; Hoffman et al. 2002). Therefore, hidden specimens appear to be relatively safe from predation.

As stated above, the bark shelters also protect the millipedes from direct insolation and thus minimise the risk of desiccation. Even if air humidity in a tropical forest remains above 85 % (Walter & Breckle 1991), this is already rather dry for many microarthropods which 'measure' air humidity in terms of saturation deficit (Stoutjesdijk & Barkmann 1992). A saturation deficit at 85 % relative humidity in a rainforest at 35 °C exceeds the saturation deficit at 65 % air humidity in a temperate forest at 20 °C (Häckel 1993). Contrary to the general impression that microclimates below a rainforest canopy are moist and constant (Beck et al. 1997), the air at the bark surface of trees can be relatively dry due to temporary high saturation deficits (cf. Prinzing & Woas 2003). Given that the air in the litter layer of a tropical forest is usually saturated with water vapour (Vannier 1970), local arthropods which seasonally leave the forest litter to climb trees are forced to cope with constraints due to relative dryness during this time. Considering the pronounced requirement for humidity in millipedes (Hopkin & Read 1992), the millipede *P. obliterata* is particularly affected. Whereas moisture is of no concern for the specimens dwelling in humid plant debris on the plantation (relative humidity: 87 ± 5 %; Chapter 3.2.1.4), conditions for *P. obliterata* are severe during the aquatic phase in the inundation forests, where the relative humidity of the habitat substrate is well below 50 % (Fig. 19). Many invertebrate species have been stated to avoid desiccation by seeking appropriate microhabitats (Warburg et al. 1984; Somme 1994). I found that, even on the plantation, the movement of *P. obliterata* was governed by humidity since specimens resettled nearby in more humid plant debris when the inhabited site dried up (Chapter 3.2.3.2). Accordingly, given that moisture declined significantly with increased height above the water line irrespective of direct solar radiation or shadow (Fig. 19, Table 6), the specimens temporarily residing on tree trunks generally selected the more humid bark microhabitats near the water (Figs 20-22). Most groups of *P. obliterata*, including the largest ones, were found at distances of approximately 10 to 20 cm from the water line, while only a few, mostly single individuals were observed at further distances (up to 150 cm). *P. obliterata* was neither detected in the upper trunk region nor in epiphytes (2 to 7 m above the ground) with the exception of one male at the Igapó site. The patchy dispersal of individuals close to water results from the extreme abiotic conditions in the trunk region. Below the water line the animals would drown (Adis & Messner 1997), whereas too far above the water line they would suffer desiccation. Similar preferences for microhabitats close to water were reported for other millipedes on local tree trunks.

Individuals of *Pycnotropis tida* linger near the water line (Vohland & Adis 1999), and some adults of *Mestosoma hylaeicum* spend the aquatic phase inside freshwater sponges above water (Adis 1992a). Even the arboricole *E. arborea* has been shown to leave the upper trunk and canopy region when forced down by insolation/drought (Adis 1984). This emphasises the relative importance of the microhabitat for an organism, compared to the macrohabitat/biotope as a whole, particularly in the structurally diverse Amazonian forests as suggested by Amedegnato (2003).

In 2002, the relative position of sampled *P. obliterata* was significantly distinct among the three different inundation forests (Table 7). The shortest average distance to the water line was observed for groups of *P. obliterata* on Marchantaria Island (MA: 13 cm), whereas those at Lake Janauarí (LJ: 20 cm) and particularly Tarumã Mirím River (TM: 34 cm) were located at higher mean altitudes. These observations can probably be attributed to spatial variability of precipitation. Evidence for this can be found in the rainfall data for Manaus registered between 1910 and 1979 that revealed considerable differences in the local precipitation patterns (Ribeiro & Adis 1984). Ribeiro and Adis (1984) recorded notably less annual rainfall for the whitewater region (i.e. MA: 1150 mm), whereas precipitation was high in the blackwater location (i.e. TM: 2157 mm). The mixedwater inundation forest at LJ may receive intermediate quantities of rainfall. In addition, the tree canopy of Várzea woodland (on MA) is comparatively open compared to the almost complete crown cover of the Igapó (at TM) (cf. Adis 2002), resulting in higher insolation and therefore a more extreme local forest climate at MA (Adis, pers. commun.). Due to the lower precipitation locally, as well as higher insolation (thus likely lower air humidity), the animals on MA were forced to dwell closer to water.

Surprisingly, the correlation between the species' relative height above the water line and the monthly precipitation (as measured at CEASA harbour near the confluence of the rivers Solimões and Negro; Chapter 3.2.1.1) was significantly negative on MA and absent at LJ (Table 9). These results suggest that, in these locations, the absolute amount of rainfall during the aquatic phase had more influence than the distribution pattern. The reason for this might have been that the precipitation data used for the correlations were rather imprecise locations, and both the local intensity, and frequency of rain events might have differed considerably on MA (Ribeiro & Adis 1984) as well as the nearby LJ (Fig. 2). In contrast, the rainfall pattern used evidently fitted better for

TM, where a significant positive correlation was observed among the relative position of specimens and the monthly precipitation (Table 9).

Choice of frequented bark microhabitats by *P. obliterata* was also influenced by macroclimatic events. The year 2003 was characterised by a moderate El Niño that resulted in a somewhat lower and also differently distributed monthly precipitation in comparison with the previous year (Fig. 16b). Most likely, the considerably lower rainfall between April and July 2003 resulted in even harsher humidity conditions for *P. obliterata*. The specimens on MA, and particularly at TM, responded to the reduced relative humidity by retreating significantly closer to the water line (MA: 9 cm, TM: 12 cm; Table 7). In contrast, the individuals at LJ did not change their relative position in 2003 (LJ: 21 cm; Table 7), but remained at the same average distance from water as in 2002. However, this is most likely due to the low local abundance of *P. obliterata* at LJ (Chapter 3.3.1) that may not have allowed for the species to successfully compete for favourable microhabitats with more frequently co-occurring millipedes. In 2003 I found no significant correlation between the relative position of *P. obliterata* and monthly fluctuations of rainfall in any of the inundation forest locations (Table 9). As stated above, this is most likely due to the fact that the data used for correlations may not reflect actual local rain patterns.

My results show that, in seasonally inundated forests, individuals of *P. obliterata* on tree trunks counteract reductions in the relative humidity by adjusting their distance closer to the water line. Although there are still only a few observations on the reaction of millipedes in a vertical humidity gradient (cf. Cloudsley-Thompson 1951), Peitsalmi (1974) reported similar changes in the vertical position of aggregated millipedes in response to humidity. Accordingly, the reaction to moisture observed for *P. obliterata* might also apply to other local millipedes that take refuge on trees close to the water line during inundation.

Interestingly, the species' height above the water line somewhat increased during the residence time on tree trunks (Figs 28a-b, 31a & 34a-b), as indicated by significant positive correlations between the residence time and relative position of *P. obliterata* at all sites in 2002, and on MA and at LJ in 2003 (Table 9). A possible explanation for this phenomenon might be the age structure of the respective populations. Due to the lack of offspring during the aquatic phase, the average age of specimens increased over this period (Chapter 3.3.2.3.1). Older stages are less vulnerable to desiccation than younger

ones (Chapter 3.3.2.3.2) and this may allow them to dwell in higher reaches from the water line.

I also observed variations in the relative position of aggregated *P. obliterata* among different tree trunks (Chapter 3.2.3.1.2). This was probably due to the characteristics of individual trees. On MA, for instance, significantly higher distances for individuals on trees 14 and 15 (Fig. 23b) may be attributed to the tree species. Both trees belong to *Vitex cymosa* (Table 1) whose bark is bast-like, very readily absorptive, and thus moistened providing a sufficiently humid microclimate also in higher reaches compared to other tree species. Similarly, both trees 8 and 10 at LJ were dead wood (Table 3) and thus comparatively more humid, allowing the millipedes to linger at significantly larger distances from the water line (Fig. 25b).

3.3.2.2.2 Gregarious Behaviour

Even the most humid, and preferred, locations on tree trunks in the seasonally flooded forests are comparatively dry (relative moisture of the habitat substrate: 35 ± 5 %; Chapter 3.2.1.4) for millipedes. While the resident individuals of *P. obliterata* seem to be more tolerant to these dry conditions than those from the upland location (at least in terms of developmental biology; Chapter 3.3.2.3.4), they still have to cope with low humidity. Interestingly, I found the local specimens to be aggregated during the day (Chapter 3.2.3.1.2), whereas those in the moist plant debris on the plantation showed no such gregarious behaviour (Chapter 3.2.3.2). Furthermore, during the terrestrial phase, the animals in the inundation forests also featured a non-aggregative mode of life, i.e. when dwelling in such an adequately moistened habitat substrate as humid dead wood (pers. obs.). Seasonal aggregation is a common phenomenon, particularly in diapausing tropical insects (Denlinger 1986), and is known from other Diplopoda retreating to ‘shelter’ sites i.e. sites with the most equalised microclimate (Vajda & Hornung 1991). In *P. obliterata* such aggregated distributions during the time spent on trees is likely to represent an additional behavioural response to avoid desiccation.

Aggregation patterns in animals are long since known to influence survival (Allee 1926), for instance by reducing the mortality of individuals that is caused by dehydration (Tadeka 1984). Here, aggregation can contribute to water conservation by limiting body evaporation as shown for isopods (Tadeka 1984), tropical beetles (Yoder et al. 1992), cockroaches (Yoder & Grojean 1997) and moths (Klok & Chown 1999).

The reason for this is that the rate of water loss in an organism is generally dependent on its size. Small animals, such as *P. obliterata*, show a large body surface-to-volume ratio, i.e. a relatively large body surface area over which water is lost by diffusion (Schmidt-Nielsen 1984). Consequently, a correlation between resistance to desiccation and body size has been demonstrated both in centipedes (Auerbach 1951) and millipedes (Crawford et al. 1986). The cuticle of most millipedes is permeable to water (Edney 1977), so diffusion through their exoskeleton is more rapid than in many other arthropods. Being a small Diplopoda species, *P. obliterata* is therefore particularly susceptible to desiccation. The observed tight body contact of individuals on tree trunks most likely reduces the relative surface-to-volume ratio and thus the loss of water by evaporation. It has already been shown for another millipede, the European *Proteroilius fuscus*, that aggregated animals can withstand desiccation for far longer, and that both the resistance to desiccation, and the tendency to aggregate increase towards the seasonal onset of hibernation (Peitsalmi 1981).

Although it has not yet been related to water conservation, gregarious behaviour during the aquatic phase has also been observed in other resident millipedes; *Cutervodesmus adisi* (Adis et al. 1996a), *Pycnotropis tida* (Vohland & Adis 1999), *Mestosoma hylaeicum* (Adis 1992a), as well as *Poratia insularis* and *Docodesmus amazonicus* (pers. obs.). Specimens from different species were even encountered in mixed aggregations (Chapter 3.3.2.2.3), indicating that the seasonal formation of aggregates is not merely related to intraspecific interactions, but is rather a general survival strategy used to avoid dehydration in the resident millipede species.

Though quiescent when aggregated by day, the millipedes are assumed to be mobile in night hours, probably dispersing to forage for food (Chapter 3.3.2.2.1). Thereafter, individuals may not return to their former residences but may rather relocate and regroup at nearby humid shelters, as observed for other (European and cosmopolitan) Diplopoda by Peitsalmi (1981) and Hamazaki (1996). This could account for the repeated occurrence of single specimens (pers. obs.) which apparently failed to re-encounter conspecifics to form groups. Naturally, this is also related to the local abundances of *P. obliterata*. Solitary individuals were found more often at both Tarumã Mirím River (TM) and Lake Janauarí (LJ), where the species is less numerous, than they were on Marchantaria Island (MA), where *P. obliterata* is dominant (cf. Figs 20-22; Chapter 3.3.1).

In 2002 the average number of individuals per group was similar at all inundation forest sites (3 to 4 individuals; Table 7). However, the distribution of group sizes was significantly distinct at TM (Chapter 3.2.3.1.2). The observed higher variance in aggregation sizes at TM, as becomes most apparent in the fourth month of flooding (Fig. 29a), is probably due to the higher local precipitation (Ribeiro & Adis 1984). Given a wider range of adequate microhabitats on tree trunks due to less severe humidity conditions at TM, the local millipedes might be more randomly distributed, resulting in fluctuating group sizes.

On MA, group size was significantly correlated with the relative position above the water line (Table 9). When further from the water, the animals coped with lower humidity by forming larger aggregations.

P. obliterata also responded to the lower relative humidity caused by the moderate El Niño in 2003 (Chapter 3.3.2.2.1) with the establishment of larger groups. In this year the species' average aggregation size at both MA (7 individuals) and TM (5 individuals) was significantly larger (almost twice as large as in the previous year: 3 to 4 individuals; Table 7), although the absolute number of individuals observed was lower (Table 8). Furthermore, the overall largest group of 150 individuals was also detected in 2003 on MA (Fig. 20), where population densities were the highest among the inundation forest locations (Chapter 3.2.1). In addition, a weak but significant negative correlation between the group sizes of *P. obliterata* and monthly precipitation was found at all locations (Table 9). This inverse relation between aggregation size and humidity was most apparent at TM, where animals may have been accustomed to more rainfall (see above), and less visible at LJ.

The local differences in the average group sizes can most likely be attributed to different local abundances of the species (see above; Table 8). The species' low population density at LJ did not suffice to establish larger aggregations in 2003. Here, the rather low increase in the average group size from 2002 (3 individuals) to 2003 (4 individuals) was not significant (Table 7). However, due to less variation as a result of the harsher climatic constraints, the distribution of aggregation sizes in 2003 was homogeneous among the three inundation forest locations (Chapter 3.2.3.1.2).

Interestingly, the number of specimens per aggregate somewhat increased during the time spent on tree trunks (Figs 26a-b, 29b & 32b), as indicated by a significant correlation between the residence time and group size of *P. obliterata* on MA in 2002, and at all locations, particularly LJ, in 2003 (Table 9). This could be either caused by

the increasing insolation (cf. Fig. 41) and thus higher temperature and increased evaporation resulting in decreasing relative humidity, and/or possibly due to the fact that more individuals encountered others over time.

These results strongly suggest that the seasonally gregarious behaviour of *P. obliterata* on tree trunks is triggered off by a decrease in relative humidity. Positive thigmokinesis, i.e. attraction to solid objects including other individuals, is a generally known behavioural adaptation to prevent water loss, for instance in isopods (Morris 1999). The animals become less active when more of their body surface is in contact with such objects, which results in the formation of groups. Since the studied aggregations of *P. obliterata* are mainly formed by conspecifics, aggregation pheromones might also be involved in the initiation and maintenance of groups. These active substances stimulate animals to form a group by encouraging other members of the same species to aggregate in a particular area (Tadeka 1984). It is likely that such aggregation pheromones have increasingly been excreted by *P. obliterata* under dry conditions.

Although the observed aggregations in the seasonally inundated forests are mainly a response to the relative dryness, this behaviour might also involve additional benefits. Millipedes, in particular polydesmids, frequently show aggregated distributions (Bhakat 1989; Bandyopadhyay & Mukhopadhayaya 1988; Hopkin & Read 1992; Tajovsky 1993). In several tropical species very dense aggregations can occur. These are often accounted for by groupings of juvenile stadia, mating patterns, feeding activities or seasonal variation in dispersion patterns (Blower & Miller 1977; Dangerfield 1993). In *P. obliterata*, only seasonality applies since its aggregations comprise both juveniles and dormant adults (Chapter 3.3.2.3.1), with foraging behaviour apparently restricted to dispersal at night (Chapter 3.3.2.2.1). In general, defense or avoidance of predators, as well as access to males are often considered as advantages of contagious distribution (Monteith 1982; Turchin 1989; Coleman et al. 2004). Correspondingly, I found that adult *P. obliterata* indeed seem to avail the presence of mating partners towards the end of the aquatic phase (Chapter 3.3.2.3.1). However, in terms of predator avoidance, such an additional benefit is likely to be exclusive to individuals under bark shelters (Chapter 3.3.2.2.1), whereas aggregations may otherwise be unfavourable in these terms because they are more easily detected. This is reflected by significantly smaller group sizes of *P. obliterata* on tree 7 on MA (Fig. 23a); it hosted a huge termite nest. The millipedes mostly avoided such trees, probably due to the resident termites defending their territory (pers. obs.). The respective tree belonged to *Eschweilera ovalifolia* (Table 1), a

hardwood species (Parolin et al. 1998) with a relatively dense bark that often forced the millipedes to hide in crevices rather than to take refuge below the bark. Due to lack of shelter and the presence of numerous termite soldiers, aggregation was disadvantageous and individuals tended to stay solitary trying to conceal themselves. This indicates additional flexibility in the behavioural response of *P. obliterata*. Other variations in aggregation sizes of the species among different trees (Chapter 3.2.3.1.2) can probably likewise be related to local peculiarities of individual tree trunks, and to different random local abundances of the species.

3.3.2.2.3 Species Interactions

During the aquatic phase in the seasonally inundated forests, I found that *P. obliterata* were repeatedly associated with individuals of other small millipede species, i.e. *Poratia insularis*, *Cutervodesmus adisi* and *Docodesmus amazonicus*. The frequency of occurrence of such foreign specimens in the aggregations of *P. obliterata* was, in part, related to the local abundances of different species (Table 8). On Marchantaria Island (MA), due to the scarcity of other species, association with the far less common congener *P. insularis* was rather an exception. In contrast, both at Tarumã Mirím River (TM) and Lake Janauarí (LJ), where other millipede species were more abundant (Chapter 3.3.1), *P. obliterata* were found comparatively often in the company of *C. adisi*, *D. amazonicus* and *P. insularis*.

In observational studies, arthropod species are often grouped into guilds according to their body size and morphology (Prinzing & Woas 2003), implying similar biotope/habitat use. Such functional grouping into one guild seems justified for four Central Amazonian millipedes; *P. obliterata*, *P. insularis*, *C. adisi* and *D. amazonicus*, as they are similar in morphology and size (Chapter 3.2.2), and overlap in their favourable microhabitats as well as activity time (Chapter 3.3.2.2.1). Species of the same guild, however, are regarded as potential competitors for the same resources.

Other terrestrial arthropods (e.g. tiger beetles) in the Central Amazonian floodplains seem to prevent interspecific competition by species-specific microhabitats, i.e. spatial segregation, and probably also species-specific prey spectra, i.e. functional segregation (Zerm 2002). This corresponds to deterministic equilibrium models, where the occupation of narrow niches allows for the co-existence of species (Holt 2001). Whereas in other studies (O'Neill 1967; Geoffroy 1981; Enghoff 1983) co-occurring

millipede species displayed niche partitioning in respect of microhabitat, separation due to seasonal migration, or body size and hence food source, such patterns do not to apply to the above mentioned millipedes. These four Diplopoda species, according to my field observations, appear convergent with respect to seasonal migrations to tree trunks and preference for certain tree species (Chapter 3.3.2.1), the use of bark shelters close to the water line (Chapter 3.2.2.2.1), gregarious distributions (Chapter 3.3.2.2.2), and, most likely, also nocturnal feeding activities (Chapter 3.3.2.2.1) during the aquatic phase, as well as univoltine life cycles (Chapter 3.3.2.3.1). Though dietary specialisation is often considered an effect of competition avoidance, and food preferences have been revealed in some millipedes (cf. Kheirallah 1979; Chapter 1.2.1.5), differential utilisation of the limited resources on tree trunks during the aquatic phase seems unlikely and local competition might arise.

One view of community ecology is that of biocoenoses in equilibrium over long periods of time, with the same group of species developing stable interactions. A more realistic view is that communities are continually challenged by invading species (Elton 1958; Simberloff 1981), with the local species often affected by the invaders (Simberloff 1981). In this context, it is remarkable that native millipedes like *C. adisi* and *D. amazonicus* appear to tolerate recently introduced species, *P. obliterata* and *P. insularis* (Chapter 3.3.1), as they are potential competitors for temporarily restricted resources, in mixed aggregations on tree trunks. In contrast, Geoffroy (1981) found that two co-existing European millipedes of equal size tended to aggregate in monospecific groups when sharing a seasonal microhabitat. However, indifferent behaviour in response to an introduction has also been reported, namely for two native millipede species in South Australia (Griffin & Bull 1995). Griffin & Bull (1995) found that the three species, which overlap broadly in activity time and preferred microhabitat, showed no difference in habitat choice or aggregating behaviour when alone or mixed, and natives commonly aggregated with the introduced species.

With regard to the four Amazonian millipedes, such a non-discriminatory gregarious behaviour may result from a secondary relevance of competition given the severe climatic conditions during the aquatic phase. This theory is supported by the view of stochastic fluctuation models that predict seasonally heterogeneous (e.g. flooded) biotopes to prevent equilibrium (climax) and thus sustain diversity, allowing for many species to overlap broadly in their niches (Holt 2001). In theory, the role of competition in stabilising communities against environmental variability may be minor, whereas

environmental fluctuations can affect the strength or presence of species interactions (Hughes et al. 2002). During the time spent on tree trunks the millipedes might be largely regulated by abiotic constraints in relative humidity and thus competition for food resources may become relatively less important, particularly since the animals do not reproduce over this period, yet another adaptation to the temporary limitations (Chapter 3.3.2.3.2). Furthermore, due to the proposed diurnal rhythm of aggregated resting by day, and scattered foraging at night, the benefit of water conservation and the limitation of food due to the presence of other specimens are chronologically detached. As a consequence, the millipedes may primarily take advantage of aggregation with other species, especially under drier conditions and/or when their own numbers are so low that specimens rather encounter foreign or mixed groups than monospecific ones.

Correspondingly, I found that the frequency of interspecific associations was affected by the relatively dry conditions caused by the moderate El Niño in 2003 (Chapter 3.3.2.1). In this year, due to the more extreme climate, the population densities of millipedes in the inundation forests appeared somewhat diminished, as indicated by the comparatively lower numbers of *P. obliterata* observed at different localities (Table 8). On MA the local abundance of the less frequent but co-occurring *P. insularis* was so low in 2003 that I only found monospecific aggregations of *P. obliterata*. In contrast, at TM as well as LJ, where co-existing species were generally more abundant (pers. obs.), both the absolute and relative proportions of foreign specimens in aggregations of *P. obliterata* were higher in 2003 than in 2002 (Table 8). In many of these mixed groups, the number of foreign specimens was therefore larger than the number of *P. obliterata* individuals. Furthermore, at both locations a significant correlation between the proportion of other specimens in the mixed aggregations and the residence time on tree trunks was found in 2003 (Table 9). This can most likely be attributed to a decline in the relative humidity over the residence time due to a decrease in precipitation (Fig. 16b) and an increase in insolation (cf. Fig. 41) during the aquatic phase. In accordance to this, a significant inverse relation between the monthly rainfall, i.e. humidity, and the occurrence of foreign specimens in groups of *P. obliterata* at LJ was found (Table 9).

All this suggests that interspecific associations arise when climatic conditions become more extreme and larger aggregations help in reducing water loss and ensure survival. The occurrence of such gregarious species interactions thus may also be referred to as a local survival strategy, given that individuals of *P. obliterata* in the moist plant debris

on the plantation did not show any species interactions (Chapter 3.2.3.2). Here, *P. obliterata* co-occurred with the small millipede *Myrmecodesmus hastatus*, but the two species were never found associated. Therefore, interspecific aggregation seems to represent another flexible, behavioural response to seasonal flooding. Due to the lower population density of *P. obliterata* at TM and LJ (Chapter 3.3.1), local individuals relied on an enhanced social behaviour with foreign specimens to establish sufficiently large aggregations and enable them to cope with the low relative humidity in 2003. Species can strongly repulse or attract one another by their pheromones, as shown for Collembola (Christensen 1980). Perhaps some aggregation pheromones exist in *P. obliterata* as well (Chapter 3.3.2.2.2). If so, most likely they are excreted by millipedes in response to drier conditions, and this could also have encouraged the formation of interspecific aggregations.

Such social species interactions for the benefit of water conservation seem particularly relevant for *P. obliterata* individuals at LJ which, in contrast to those on MA and at TM, did not retreat closer to water, nor formed larger groups to cope with the reduced humidity in 2003, probably due to the lower local abundance (Chapters 3.3.2.2.1 & 3.3.2.2.2). Here, a significant inverse relation between the presence of foreign specimens in mixed groups with *P. obliterata* and the relative position of groups above the water line was observed in 2002 (Table 9). This can probably be explained by the observation that all considered millipede species preferred microhabitats close to water; thus encounters of, and association with, other individuals in these areas was more likely. However, in 2003, the correlation between the proportion of foreign specimens in aggregations of *P. obliterata* and the relative vertical position of groups was significantly positive. Possibly, as a result of increased competition for microhabitats near the water due to reduced relative humidity, foreign specimens were not tolerated in aggregations at the favoured sites, while at greater distances from the water line, where abiotic conditions were worse, the present, probably less competitive individuals of different species readily associated.

3.3.2.3 Life Cycle and Postembryonic Development

For migrating species such as *P. obliterata*, the most important complex adaptation involves the relation between migratory behaviour and the schedules of reproduction and mortality (Rankin & Singer 1984). Migration allows the choice of, where to breed,

and its relation to births and deaths determines the life history strategy (Dingle 1985). The life cycle of most terrestrial arthropods is closely adjusted to seasonal changes, with development and reproduction being limited to specific periods of the year that alternate with periods of dormancy, i.e. inactive periods (Tauber et al. 1986; Schäfer 1992). Both migration and dormancy are thus methods that animals have evolved to meet changes in their biotopes/habitats (Southwood 1962).

3.3.2.3.1 Life Cycle in the Field

In Central Amazonia, the development of specific reproduction cycles by terricolous arthropods is related to the transition from upland to floodplain forests (Adis et al. 1988). Numerous species living in upland forests show a plurivoltine life cycle, i.e. continuous reproduction, while those in seasonally flooded forests have acquired an annual periodicity of reproduction, i.e. univoltine life cycles, characterised by the absence of offspring during the aquatic phase (Adis 1997). For the latter species, the main reproduction occurs on the forest floor during the subsequent terrestrial phase. Such a synchronisation of reproduction in the soil with the non-inundated period has been observed for resident millipedes such as *Cutervodesmus adisi* (Adis et al. 1996a), *Pycnotropis tida* (Vohland & Adis 1999), *Mestosoma hylaeicum* (Adis 1992a) and probably *Prostemmiulus adisi* (Mauriès 1984), as well as *Poratia insularis* and *Docodesmus amazonicus* (pers. obs.). Even the non-migratory, flood-resistant millipede *Myrmecodesmus adisi* (Adis & Messner 1997) shows such a univoltine life cycle in the inundation forest.

Accordingly, I found that the *P. obliterata* population on the local upland plantation at CPPA/Embrapa (EB) reproduced continuously, with both immature stages and adults occurring throughout the observation period (Fig. 41). In contrast, the reproductive cycle of *P. obliterata* populations in the three different inundation forests is synchronised with the annual flooding. A significant increase in older stages and a lack of eggs, recent hatchlings and succeeding juvenile stages were observed during the aquatic phases in 2002 and 2003 (Figs 35-37; Chapter 3.2.4.1.1), indicating a univoltine life cycle in the seasonally flooded biotopes. The majority of *P. obliterata* individuals spent the aquatic phase in the 6th and mainly subadult stages. A similar dominance of advanced juvenile stages during inundation has been reported for another local millipede, *C. adisi* (Adis et al. 1996a). Most likely, these stages are more resistant to the

harsh, dry conditions prevailing on tree trunks (Chapter 3.3.2.2.1) than younger ones. David & Vannier (2001) found that resting subadults of the European millipede *Polydesmus angustus*, for which desiccation might also be an important mortality factor in the field, survived dehydration stress far longer than other immature stages. One reason for this might be that subadults did not moult until September, thus avoiding extra water loss during moulting (Hopkin & Read 1992; David & Vannier 2001). Ecdysis (i.e. moulting) is the most sensitive period during the development of millipedes (Hopkin & Read 1992). Most likely, this is reflected in the higher mortality of premature *P. obliterata* from MA (average mortality: 34 %) compared to those from EB (average mortality: 10 %) in enclosures (Fig. 49a; Table 15; Chapters 3.1.6.2 & 3.2.5.2), since the proportion of 6th stage immatures compared to subadults was higher in *P. obliterata* from MA (pers. obs.), i.e. the respective individuals had to perform more phases of ecdysis. As to water conservation and thus an increased resistance to desiccation by a delayed ecdysis, such a benefit may apply to subadult millipedes on tree trunks in floodplain forests, since the majority of these did not moult before the end of the aquatic phase. While in *C. adisi* advanced juvenile stages only reached maturity after their return to the ground (Adis et al. 1996a), a considerable fraction of subadult *P. obliterata* turned adult at the end of inundation, i.e. prior to the recolonisation of the forest floor. This pattern became most apparent on Marchantaria Island (MA), where the species appeared to be most frequent (Chapter 3.3.1) and more than 80 % of all individuals encountered during the last month of inundation in 2002 had become adult (Fig. 35). Hence, reproduction in *P. obliterata* can probably be resumed more rapidly in the subsequent terrestrial phase.

The sex ratio in *P. obliterata* was balanced and did not differ significantly during the aquatic phase or among years and sample sites, including the upland plantation (Chapters 3.2.4.1.1 & 3.2.4.1.2). Still, in the inundation forests the ratio was slightly but not significantly biased towards female juvenile and adult stages (Figs 38-40). Similarly, a probable predominance of females on the forest floor was observed for the local *C. adisi* (Adis et al. 1996a). Adis et al. (1996a) suggested that this might help in compensating for a possible decline in population density due to flooding by a faster establishment of subsequent generations in the non-inundated period.

By the end of the aquatic phase in 2003, the proportion of females with mature and immature eggs increased significantly from August to September (Fig. 43; Chapter 3.2.4.2.1). In September, closer to the terrestrial phase, all dissected females from the

inundation forests carried eggs, whereas females with as well as without eggs were found on the upland plantation, where animals reproduced continuously (Fig. 43; Chapter 3.2.4.2). In the inundation forests the average number of both mature and immature eggs per female increased significantly towards the terrestrial phase, with the quantity of mature eggs being higher than the number of immature eggs, whereas an opposite trend was observed in females from the upland plantation (Fig. 44; Chapters 3.2.4.2.1 & 3.2.4.2.2). In September, close to recolonisation of the forest floor, the numbers of mature and immature eggs were thus significantly higher in females from the inundation forests compared to those from the upland plantation. All these observations suggest that females in the inundation forests increasingly built up eggs which matured towards the end of the aquatic phase, but were not yet laid. A similar pattern was also observed in females of the univoltine millipede *P. tida*, where the highest quantities of mature eggs were also found at the end of the aquatic phase (Vohland & Adis 1999). Eggs in local females of *P. obliterata* are therefore likely to mature only close to trunk descent, with oviposition being restricted to the terrestrial phase after females returned to the forest floor. On the upland plantation, copulating adults were recorded throughout the observation period in both 2002 and 2003. In contrast, although adults of both sexes occurred all along, mating in the inundation forests was only observed sporadically at the end of the aquatic phase (a total of seven copulations for all locations in both years; Chapter 3.2.4.2.1). These copulations, however, do not necessarily imply reproduction in the trunk region, since fertilisation in millipedes is not coupled to copulation. It is important to recognise a time lag between insemination and fertilisation. The process of insemination occurs during mating when the male inserts a packet of sperm into the spermathecae of the female (Barnett et al. 1993). The sperm is then stored in these seminal receptacles, and eggs are not fertilised until just before laying (Blower 1985; Hopkin & Read 1992). Some females probably avail the presence of mating partners during the temporarily gregarious mode of life (Chapter 3.3.2.2.2) to copulate close to the end of the aquatic phase, enabling immediate oviposition after their return to the ground in the terrestrial phase (cf. dormancy in Chapter 3.3.2.3.3). Snider (1981) reported that females of the millipede *Polydesmus inconstans* appear to be able to get enough sperm from one mating to fertilise all their eggs, encompassing seven to eight ovipositions. Further evidence for the abstinence from mating by sexually mature adults of *P. obliterata* until the end of the aquatic phase is given by experiments performed on females sampled from April to August in 2003

(Chapter 3.1.6.2). All females copulated with a paired male before laying eggs, whereas females which were kept single or with an immature male did not oviposit.

Females of *P. obliterata* in the inundation forests may produce an attractive signal (e.g. a mechanical or chemical signal; cf. Haacker 1974) when intending to mate close to trunk descent. Support for this has been seen in studies of the tropical beetle *Stenotarsus rotundus*, which shows a similar pattern of resuming reproduction after dormancy during a harsh season than here proposed for *P. obliterata*. Near the end of the dry season, females in the aggregation initiate egg maturation (Wolda & Denlinger 1984) and, at the onset of the rainy season, copulate and then disperse from the aggregation to lay their eggs (Denlinger 1986). Here, the precise stimulus that initiates copulation is assumed to be increased locomotory activity (Tanaka et al. 1987b).

Phenology of *P. obliterata* in the field is apparently also influenced by variations in the flood pulse due to macroclimatic events. In 2002 only few individuals of developmental stages younger than the 5th were found on tree trunks at the beginning of the aquatic phase, i.e. shortly after trunk ascents, whereas in 2003 the proportion of younger developmental stages (stage 3 to 4) was higher (Figs 35-37). This is likely to be explained by later forest flooding in 2003 due to the moderate El Niño (Figs 17 & 18). Thereby, the preceding terrestrial phase was extended for approximately one month, allowing for the litter-dwelling animals to produce additional offspring, i.e. hatchlings from formerly laid eggs, as well as their further development to juvenile stages. This agrees with the observation of Adis (1997) that in some local species, where trunks ascents are normally represented by adults, juvenile stages occurred as well in years with El Niño events, e.g. in the millipede *P. tida*, where two overlapping generations seem to mix in this situation (Vohland & Adis 1999). Although the seasonal rhythmicity in age structure of local *P. obliterata* is thus affected by environmental variation, maturation occurred each year mostly at approximately the same time, i.e. at the end of the aquatic phase (Chapter 3.2.4.1.1).

The hypothesis that an extended terrestrial phase, due to a prolonged reproductive period, may lead to a higher proportion of juvenile stages in *P. obliterata* is also reflected in the local variation of its age distribution. The sample sites at Lake Janauarí (LJ) as well as Tarumã Mirím River (TM) were situated at higher average altitudes than the site on Marchantaria Island (MA; Chapter 3.2.1.3). In addition, the sample site at LJ was locally much more heterogeneous in terms of elevation than the other two, resulting in large variations among flood periods of individual sample trees (up to 100 days;

Chapter 3.2.1.3). Accordingly, the sampled millipedes at LJ were perspicuously younger than those collected at the other two locations (Chapter 3.2.4.1.1). For instance, whereas age distribution in the last months of flooding in 2003 resembled that of the previous year for *P. obliterata* on MA and at TM, many 6th and some 5th developmental stages were still encountered at LJ.

On the upland plantation, the number of recorded *P. obliterata* per month decreased significantly during the observation period in 2002 (Chapter 3.2.4.1.2). This might be due to the beginning of the dry season, as more juvenile stages seem to occur in months of more rainfall (Fig. 41). However, results did not indicate any significant correlation between local precipitation or insolation patterns and the phenology of *P. obliterata* in both 2002 and 2003 (Fig. 41; Chapter 3.2.4.1.2). The high moisture of the plant debris on the plantation seems to effectively shelter the millipedes they are inhabited by from external climatic conditions.

3.3.2.3.2 Evolution of the Univoltine Life Cycle

The most prominent life history trade-off involves the cost of reproduction (Stearns 1989). It has two major components, cost paid in survival and cost paid in future reproduction. Stearns (1989) emphasised the importance of intergenerational trade-offs, i.e. trade-offs between a female's reproductive effort and the probability that her offspring will survive to the next season. He stated that both the reproductive investment of a female and the survival of its juveniles are phenotypically plastic traits. Any change in a female's allocation strategy is likely to have implications for juvenile mortality (Stearns 1989). This can lead to a trade-off between current and future reproduction. Many organisms are capable of reducing or delaying their reproductive output when the conditions for potential offspring are unfavourable (Boyce & Daley 1980).

For terrestrial invertebrates in the floodplain forests, temporarily suspending the reproductive effort during the rather adverse aquatic phase may be an adaptation to minimise the costs associated with reproduction, since chances for a successful reproduction are very low anyway. As observed for *P. obliterata* by Wilck (2000), cultured females of an inundation forest population were able to survive for almost a year when kept single, but died after a maximum of three months if they had copulated. Most likely, the longevity of mating females was significantly affected by subsequent

reproductive investments. Under the adverse conditions (e.g. relative dryness, limited resources; Chapter 3.3.2.2) prevailing on tree trunks, where animals take refuge during flooding, this effect on female survival is likely to be even more severe. Besides this, any eggs or hatchlings emerging during this period would certainly suffer high mortality. Millipede juveniles are more vulnerable than adults due to their small size and softer exoskeleton (Hopkin & Read 1992). They represent an easier prey for predatory arthropods in the trunk region (Chapter 3.3.2.2.1) and lose water generally faster than adults, thus being less resistant to desiccation (O'Neill 1969; Lewis 1974; David & Vannier 2001; David et al. 2003b). In the laboratory (Chapters 3.2.5.1 & 3.2.5.2) I found the mortality of offspring in *P. obliterata* to be particularly high under drier conditions. Accordingly, with the relative dryness on tree trunks being an important mortality factor even for adults, juveniles of the local millipede *Pycnotropis tida* were not able to survive the aquatic phase (Vohland & Adis 1999). Since their survival probability is higher in humid places, early juvenile stages of millipedes usually only appear at sites with the most equalised microclimate and moist conditions (Peitsalmi 1981; Vajda & Hornung 1991). Such humid shelters, however, are not encountered on tree trunks, nor do they exist even close to the water line. Oviposition below the water line is not an option either. Firstly, the surrounding water may reduce oxygen penetration into eggs and inhibit embryogenesis (cf. Bercovitz & Warburg 1988). Secondly, millipede offspring (up to the 3rd stage) are also highly susceptible to an excess of water (Baker 1978).

In the floodplains, univoltine life cycles synchronic to annual inundation thus might have evolved in immigrant terrestrial arthropods (Chapter 3.3.2.3.1) such as *P. obliterata* due to selection caused by seasonal juvenile mortality. Age-specific mortality in a population of overlapping generations can, in theory, result in shifts in the age distribution (Galvani & Slatkin 2004). Charlesworth (2003) suggested that changes in the mortality of early life stages have an even greater effect than those that occur later in life. Furthermore, periodic mortality is believed to cause comparatively higher selection intensity than continuous mortality, with the age structure of an affected population continuing to change even after selection ceases (Galvani & Slatkin 2004). Accordingly, Reznick et al. (1990) found that reduced juvenile survival in guppies selected for later maturation and lower reproductive effort as well as less offspring and caused significant, heritable life-history evolution after 30 to 60 generations.

3.3.2.3.3 Regulation by Maternal Effects

Periods of dormancy involve either quiescence or diapause. An essential difference between the two types of dormancy is that quiescence is an response to adverse conditions that rapidly reverses after a return to favourable conditions, whereas diapause anticipates adverse conditions and is not immediately reversible (Tauber et al. 1986; Schäfer 1992).

In many arthropods, diapause is the primary seasonal adaptation (Tauber et al. 1986). There is evidence for a widespread diapause existence among tropical insect species, including those exposed to periodic inundations (Denlinger 1986). In his review, Denlinger (1986) described diapausing insects to retreat to sites that provide protection and buffering of the physical environment, just as observed for *P. obliterata* residing on tree trunks in the floodplain forests (Chapter 3.3.2.2.1). Furthermore, he stated that non-stationary aggregations that move horizontically in response to humidity cues, a pattern also observed for *P. obliterata* during the aquatic phase (Chapter 3.3.2.2.2), appear to be common during the adult diapause of tropical invertebrates (e.g. Coleoptera, Lepidoptera and Hemiptera).

In millipedes, although periods of dormancy have been reported (Hopkin & Read 1992), it is generally unknown whether these are diapauses or quiescences. According to my own observations, the univoltine life cycle of *P. obliterata* in the inundation forests involves an optional adult dormancy, i.e. quiescence, since reproduction resumed rapidly under apparently more favourable conditions in culture (Chapter 3.2.5.2). Furthermore, the cultured adults from both a univoltine inundation forest population and a plurivoltine upland population immediately ceased to reproduce when forced to take shelter on bark or container walls in response to experimental flooding (Chapter 3.2.5.1). Hence, the species appears to be able to track environmental changes by adjusting its reproductive behaviour, clearly a profound selective advantage.

Selection experiments with tropical insects suggest a capacity for rapid adaptation with respect to dormancy traits (Denlinger 1986). Genetic variation provides a framework for life cycles that are then modified by developmental and environmental variation. The pronounced genetic diversity found in *P. obliterata* (Chapter 4.3.2) could form the basis of the observed local flexibility in its life cycle. If the environment is predictable, external cues can trigger off a switch between the alternative forms best suited for prevailing conditions (Scott & Dingle 1990). Since in the floodplain forests secondary,

abiotic factors (Chapter 3.1.2.1) indicate the advance of inundation and can be monitored, plasticity might be favoured because it allows effective adjustments to the changes. In a colonising species such as *P. obliterata* the evolved plasticity with adaptive responses in each environment encountered would clearly be advantageous.

According to Groeters & Dingle (1987, 1988), one way the constraining effects of genetic correlations across environments can be circumvented is by selection for environmentally cued maternal control of the characteristics of offspring. Maternal effects can thereby provide considerable flexibility in the life cycle because the female can influence future performance of its offspring in temporally heterogeneous environments (Groeters & Dingle 1987, 1988). These maternal effects can potentially affect the whole suits of life history traits, since they appear to influence the programme of juvenile hormone production throughout offspring lifespan, as shown for insects (Nijhout & Wheeler 1982).

Whereas some millipedes are believed to synchronise their moulting period (Bhakat et al. 1989), I found the time of postembryonic development in reared *P. obliterata* to be highly variable between different rearing boxes (cf. high values of standard deviation in Table 12). Different rates of development have also been reported for cultured individuals of the local *P. tida*, with slow ones taking up to one year longer to reach maturity (Vohland & Adis 1999). As suggested by Vohland & Adis (1999), a high degree of internal variability is possibly present in *P. obliterata* and *P. tida* to widen their survival options.

According to Wilck (2000) and Adis et al. (2001), specimens of *P. obliterata* from an upland plantation at CPPA/Embrapa and from floodplain forests at Nauta, Peru as well as Tabatinga, Brazil showed no significant difference in the duration of postembryonic development, which took an average of 70 days. However, these animals had been reared for several generations under the constant, optimal conditions of a European laboratory. The observations in culture thus may not reflect development in the field because here additional, yet unknown factors are probably vitally important, e.g. in respect of maternal effects. Therefore, I conducted experiments on the reproduction of *P. obliterata* directly collected in the field (Chapter 3.1.6.2). Interestingly, I found the time for postembryonic development to differ significantly between animals from the upland plantation at CPPA/Embrapa (EB) and the local inundation forest of Marchantaria Island (MA). Both maturation and development were comparatively faster in offspring from the inundation forest population MA (cf. Tables 12 & 13). Relative to

MA, the average development of juvenile stages from the upland population EB was slowed down by almost one developmental stage from the 2nd to 5th month of the observation period (six months). Even more important, the first immature stages from MA reached adulthood in the 3rd month of observation, whereas offspring from EB took no less than six month to turn adult. Variation in the rates of development and the onset of maturity between contrasting biotopes has also been reported for two European millipedes which, similar to *P. obliterata*, cover a wide range of biotopes (Fairhurst 1974). Growth variation in millipedes has generally been ascribed to environmental factors and, in part, to alternative growth pathways (Peitsalmi 1981). According to Peitsalmi (1981), the variability in the maturation rate recorded in several other millipede species (Blower 1970a; Blower & Miller 1974; Fairhurst 1974) can probably be explained in a similar way.

The lack of congruence between the laboratory study of Adis et al. (2001) and my own enclosure study strongly suggests that the observed variations in the developmental rate of offspring are not permanent but field-derived. Apparently, environmental factors which the respective parents have been exposed to in the different biotopes induced a different mode of development in their offspring, most likely due to maternal effects. These might involve hormonal regulation that would influence offspring development for longer periods (Nijhout & Wheeler 1982). Alternatively or additionally, it is possible that the eggs of females from the floodplain populations comprised more resources, e.g. richer yolk, that may enabled a comparatively faster development and thus an earlier maturation in juveniles from MA. It is generally believed that young millipedes only start feeding after moulting to the 3rd stage, implying that the first two juvenile stages have to rely entirely on yolk for early development (Hopkin & Read 1992). Still, such an abstinence from feeding is not obligatory for the early juvenile stages of *P. obliterata* in culture, where I observed them to nourish on protein-rich fish food (cf. Chapter 3.1.6.2). However, this might solely apply to the field, where only less nutritive food sources are available. The better food quality for several generations in culture could also be the cause of the generally faster development observed in the study of Adis et al. (2001).

In the enclosures, I found that, similar to observations in the field, reared *P. obliterata* from the upland population EB reproduced continuously during the entire observation period (six months), while animals from the inundation forest population MA ceased to reproduce after the 3rd month as the latest (Table 14). Thereby, pairs of *P. obliterata*

from EB outnumbered those from MA in the quantity of both egg clutches and hatched offspring. Although single clutches of the inundation forest population MA often comprised more eggs (five to 15 eggs) than those from the upland population EB (five to eleven eggs; Chapter 3.2.5.2), the total numbers of eggs laid was higher in the latter. The maximum number of clutches observed per pair was four in MA and eight in EB, resulting in a maximum number of 28 and 55 first juvenile stages, respectively (Chapter 3.2.5.2). The highly variable numbers of eggs and ovipositions may again reflect instability of the environment, with the internal variability in *P. obliterata* ensuring the species persistence.

All these findings suggest alternative reproductive strategies in the two populations from different biotopes. Females from the upland population EB, which is plurivoltine in both field (Chapter 3.3.2.3.1) and enclosures, produce more offspring and for a longer period, probably investing less in single eggs. Their juveniles thus requiring a comparatively long time for development is not an issue in the field, since their habitat is not a temporary one. In contrast, females from the inundation forests, which are univoltine in the field (Chapter 3.3.2.3.1) and show a constrained reproduction period in the enclosures, have less offspring and are likely to invest more resources into single clutches. Presumably due to such higher maternal investments, the resulting juveniles are able to develop and mature more rapidly. The relatively fast postembryonic development could be a prerequisite for *P. obliterata* to inhabit floodplain forests, given that the reproductive period is locally restricted to only 5 to 7 months. This might allow for the establishment of two succeeding generations in the terrestrial phase and thus enables the species to cope for missing reproduction during the aquatic phase. As has already been proposed for other millipedes, one of them the local *P. tida*, the species' distribution range is governed by a minimum development time required from oviposition to hibernation (Halkka 1958; Vohland & Adis 1999).

3.3.2.3.4 External Cues for Reproduction and Development

Tropical invertebrates experience a rich variety of biotic and abiotic factors. The environmental cues for dormancy in tropical insects have been identified only for some species and include temperature, photoperiod, rainfall, nutrition and airborne chemicals (Denlinger 1986). Fewer studies have focussed so far on external factors that control the dormancy in millipedes (e.g. David et al. 2003a). Fujiyama (1996) concluded that

temperature is the main factor regulating the life cycle of a Japanese species. Bercovitz & Warburg (1988) further suggested temperature to affect oocyte development and the onset of egg-laying in an Israeli millipede. Temperature is also involved in the control of regional reproductive strategies in *P. obliterata*. Adis et al. (2001) obtained males from eggs of obligatory parthenogenetic females from a European hothouse (24/24 °C) by keeping the females at higher temperatures (29/24 °C). Furthermore, oviposition in some millipedes depends on soil moisture, since dry soil did not stimulate egg-laying or caused a decline in clutch size while females preferred to oviposit in moist soil (Bercovitz & Warburg 1988).

The precise regulation of univoltine life cycles in most terrestrial invertebrates, including millipedes, of Central Amazonian floodplains still remains obscure. Cues for external control have, so far, only been investigated in detail in the tiger beetle *Pentacomia egregia*. Amorim et al. (1997) found the presence of soil to be the primary factor inducing oviposition, whereas decreased variation of day and night temperature due to the buffering effect of the water body acted as a synchroniser for gonad dormancy and delayed maturation. In the millipede *Pycnotropis tida*, however, a univoltine life cycle during flooding was caused by the mortality of juveniles, mainly a result of the dry conditions on tree trunks (Vohland & Adis 1999).

Factors implicated in the regulation of dormancy in resident *P. obliterata* thus may also involve mean air temperature and adequate oviposition sites characterised by the presence of soil and sufficient moisture. In this species, soil is even more likely to be mandatory for reproduction, since the construction of egg chambers is prerequisite to egg-laying (Chapter 1.2.1.4) and most millipedes ingest earth and then use faecal material to manufacture these chambers (Hopkin & Read 1992). Another factor worth considering is the availability of resources that are certainly restricted in the trunk region, even more so due to the presence of potential competitors (Chapter 3.3.2.2.3). Food quality has been shown to affect female fertility in some millipedes (David & Celerier 1997).

Based on experiments conducted with specimens of *P. obliterata* brought from both a local inundation forest (MA) and an upland plantation (EB), however, neither a decreased variation of day and night temperature (24/24 °C), nor the lack of soil, adequate moisture and sufficient resources appears to trigger off the observed reproductive abstinence in the inundation forests (Chapter 3.2.5.2; for experimental design see Chapter 3.1.6.2 and Table 5).

A constant temperature of 24 °C did neither effect gonad dormancy, nor did it prevent maturation and subsequent reproduction in immature millipedes sampled from both populations. Besides, it had no significant influence on the mortality of both premature and adult individuals (Fig. 49a-b; Tables 15 &16).

Despite the drier conditions, pairs from both populations kept on bark pieces reproduced almost (except for 7.5 %, i.e. six out of 80 pairs) as readily as those maintained on earth. Similarly to a European polydesmid (Voigtländer 2000), female *P. obliterata* were able to build egg chambers from any material available in culture, i.e. earth, bark, plaster and filter paper (Chapter 3.2.5.2). Hence, construction material is not a limiting factor during flooding, nor does the presence of soil appear to be prerequisite to oviposition in *P. obliterata*. Although I cannot exclude that mere handling, i.e. transport of sampled millipedes from the field to laboratory in boxes with moist soil (Chapter 3.1.2.1), might have stimulated reproductive behaviour in adults, this does not apply to immature individuals which were then kept exclusively on bark and likewise reproduced after they had matured (Chapter 3.2.6.2). Even though they reproduced, the mortality in adults was significantly higher for the millipedes that kept on bark instead of soil when no moist plaster was added to provide for adequate humidity (Fig. 49b; Table 16). The average mortality then increased from < 20 to 61 %. This adverse effect resulted from the relative dryness and not caused a calcium deficit due to the missing plaster, since ecdysis in *P. obliterata* ceases with maturation (Chapter 1.2.1.4) and the adults thus show no special requirements for calcium. Juvenile millipedes were also particularly affected by the drier conditions (Chapter 3.2.5.2).

When millipedes collected from the field were kept on either habitat substrate (earth/bark) without any supplementary food (i.e. fish food; Chapter 3.1.6.2), the surviving females still oviposited but the mortality was extremely high, i.e. adding up to 90 % in adults (Fig. 49b, Table 16) and even higher values in their offspring (almost 100 %; Chapter 3.2.5.2).

As documented by the high mortality rates, low humidity and food deprivation in the last-mentioned treatments were apparently more severe than conditions in the field (during the aquatic phase). On the one hand, the fact that surviving females still mated and laid eggs may confirm that neither relative dryness nor a lack of food prevent oviposition in *P. obliterata* on tree trunks. On the other hand, as chances for survival and future reproduction were minor due to the deleterious conditions in the experiments, the respective females might only have invested in their last possible reproduction, a

situation that does not apply to the field, where the survival of adults appears less strongly affected (Chapter 3.2.4.1.1).

Possibly only several unfavourable factors combined provoke quiescence in *P. obliterata*, as observed in the aquatic phase in floodplain forests (Chapter 3.3.2.3.1) and during experimental flooding (Chapter 3.2.5.1). Alternatively, there might be another external cue not yet considered or sufficiently restricted during my experiments. It is not a trivial task to detect the precise stimulus that triggers offs or delays reproductive behaviour in a species, this being the reason why regulation in the majority of local arthropods still remains unknown. A potential factor that might be involved is photoperiod (Denlinger 1986; Tanaka et al. 1987a). David et al. (2003a) showed that adult diapause and possibly also subadult aestivation in a French polydesmidan millipede were photoperiodically induced, even though all Polydesmida are known to be blind. However, given that breeding specimens in my experiments were, for the most part, maintained in outdoor enclosures (Chapter 3.1.6.2), i.e. under the same daylight regime as quiescent individuals in the field, a photoperiodic regulation of the dormancy in *P. obliterata* is unlikely. Another hypothesis is that avoidance of reproduction during the aquatic phase may be explained in terms of predation risk, since predatory arthropods also migrate to and dwell in the trunk area over this period (Chapter 3.2.3.1.1). Furthermore, while pairs in my experiments on reproduction were kept single, intra- or interspecific competition for limited resources and more adequate oviposition sites on tree trunks may play an important role in the field.

Although the tested external factors had no effect on the quiescence in *P. obliterata*, they affected the maximum length of the reproductive period (Table 14). When kept at a constant temperature of 24 °C, females from the upland population EB did not reproduce continuously during the entire observation period (six months) but they only produced offspring up to the 2nd or 3rd month. Their reduced breeding period then equaled that of females from the inundation forest population MA. Here, reproduction was generally restricted to a period of mostly three months, regardless of treatment. Most likely, the breeding period in these females was already attuned to the harsher conditions encountered during the aquatic phase (see alternative reproductive strategy in Chapter 3.3.2.3.3). Since the upland population EB lacked such pre-adjustments, the continuity of reproduction in the respective females was acutely influenced by the decreased temperature. Similarly, the rearing of animals on bark instead of soil only affected the reproductive output in the upland population EB, i.e. by reducing the

breeding period to a maximum of four months due to the drier conditions. When maintained without plaster as an additional moisture source, the reproductive period of females from both populations was not further diminished but rather, in part, extended for one month (Table 14). Most likely, this can be ascribed to additional resources, given that the moist filter paper used to replace the plaster was treated as another food source (see below). Accordingly, a complete lack of supplementary fish food influenced the fecundity of females from both populations, reducing the reproductive period to a maximum of only two months due to the harshly restricted resources. This implies that food limitations may play a major role in the local regulation of reproduction in *P. obliterata*.

Furthermore, the investigated external factors significantly influenced the postembryonic development of resulting offspring in *P. obliterata* during the observation period (six months; Table 11). The respective effects on the developmental rate of juveniles were population-specific, i.e. differed between specimens from the contrasting biotopes, floodplain forest MA and upland plantation EB (Figs 47 & 48).

When maintained at a constant temperature of 24 °C, the average development of juveniles from both populations slowed down by approximately one stage compared to the control at higher, ambient temperature (Fig. 48; Tables 12 & 13). These findings correspond to other observations in the field (Fairhurst 1974) and laboratory (Kinkel 1955; Halkka 1958) that higher temperatures accelerate the development of millipede juveniles.

Immature stages were generally slower in growth when kept on bark instead of soil (Fig. 47), probably due to the lower humidity, but offspring from the inundation forest population MA (Table 12) were less affected than those from the upland population EB (Table 13). On bark, the average development was comparatively delayed for approximately 0.5 stages and one stage in juvenile stages from MA and EB, respectively. Here, the first individuals from MA reached adulthood only in the 5th month compared to the 3rd month in the control, whereas juveniles from EB generally took no less than six months to turn adult (Chapter 3.1.6.2). Hence, although both development and maturation were also affected, specimens from the inundation forest population MA were more tolerant to the dry conditions on bark than individuals from the upland population EB.

In the treatment without plaster as an additional source of moisture, the development of juveniles was more rapid, particularly for offspring from the upland population EB (Fig.

48; Tables 12 & 13). Here, immature stages were on average more than one stage older than in the control, and the first individuals reached adulthood already in the 4th month compared to the 6th month in the control. The juveniles originating from MA were mostly approximately 0.5 stages older compared to the control, but the first adults only occurred in the 4th month, i.e. one month later than in the control. The accelerated growth of juveniles could have been due to the fact that the moist filter paper replacing the plaster was readily accepted as additional food consisting of cellulose. Although there is some controversy as to whether millipedes are capable of digesting cellulose, cellulase activity has been observed in a few species, most likely derived from gut microorganisms, and is probably due to the animals' habit to eat their exuvia (Marcuzzi & Turchetto-Lafisca 1977; Beck & Friebe 1981; Taylor 1982; Hopkin & Read 1992). *P. obliterata* in cellulose-rich environments such as banana stems on the plantation EB could have supported a microfauna more active in cellulose breakdown to use this resource more efficiently. That might explain why they gained a higher benefit from the supplementary cellulose than did *P. obliterata* from the inundation forests. No lack of calcium (necessary for the formation of an exoskeleton after ecdysis; cf. Hopkin & Read 1992) became apparent in this treatment. Given that some millipedes from both local inundation and upland forests can extract enough calcium for the production of a calcium-rich cuticle even from wood of very low calcium content (Vohland et al. 2003), the tiny *P. obliterata* in my experiments were evidently quite able to do likewise from the soil and bark provided.

The effect of food deprivation (in the absence of fish food) on the development of juvenile millipedes was most severe, since almost all offspring died premature, with only very few subadults from the upland population EB surviving up to the 5th month (Fig. 48; Tables 12 & 13). Again, that emphasises the relative importance of available resources for reproduction in *P. obliterata*. Possibly, food limitations on tree trunks could have an even more deleterious effect on the survival of millipede juveniles than did the relative air dryness.

3.3.3 Synopsis

The study revealed local variation in the relative abundance of *P. obliterata* in Central Amazonia. These biotope-specific densities are likely to be influenced by the species' colonisation pattern, i.e. distribution along the rivers and introduction by man, as well as

on other factors such as the availability of, and competition for, local resources. Sites inhabited by other species hold limited resources for those species that may follow, and abundance of resources appears to be an important factor in both settlement and population dynamics of *P. obliterata*.

The terricolous *P. obliterata* has become adapted to annual inundation in the floodplain forests by means of survival strategies that are based on avoidance, i.e. a seasonal migration to tree trunks in direct response to the rising waters. The resulting arboreal way of life during the aquatic phase involves ethological and physiological adaptations to avoid dehydration. The local specimens, which were shown to be more tolerant to inundation (Wilck 2000), also appear more tolerant to the drier conditions compared to the specimens from upland sites. They select the most humid microhabitats near the water line and live in aggregations to reduce water loss. In addition, a response to reductions in relative humidity (due to a moderate El Niño in 2003) by adjusting their distance closer to the water line and by forming larger groups. Due to the low densities of single species in tropical forests (cf. Schaller 2005), individuals repeatedly form mixed groups with other small millipede species. Such species interactions occurred more frequently in the drier year 2003, suggesting a secondary relevance of competition given the acute constraints in moisture.

Field observations point to two alternative reproductive strategies in *P. obliterata* from non-flooded and seasonally flooded biotopes. Whereas the species showed a plurivoltine life cycle, i.e. continuous reproduction, on uplands, its reproduction was univoltine and limited to the terrestrial phase in floodplains. This univoltine life cycle may be an adaptation to minimise costs associated with breeding since the survival probability of offspring is low on tree trunks. Experiments showed that the development of offspring from floodplain females is relatively faster. This may enable the establishment of two subsequent generations in the terrestrial phase that is restricted to 5 to 7 months.

Population differences in migration or other traits can result from either genetic or environmental causes (cf. Dingle 1991). Experiments suggest the latter since vertical migration and discontinuous reproduction can also be observed in individuals from an upland population when exposed to flooding. In addition, experiments demonstrated that the univoltine life cycle in the floodplains is based upon optional adult dormancy (quiescence) in *P. obliterata*, and that reproduction is rapidly resumed after a return to

more favourable conditions. The external cue ('trigger') for its regulation still remains to be clarified.

Further, the experiments suggest a higher disposition for both vertical migration and restricted reproduction in specimens from floodplain forests. The comparatively faster development rate in offspring originating from this biotope, however, appears to be field-derived and not genetically inherited. Environmental factors to which females were exposed in the field may induce a different mode of development in their offspring, suggesting a maternal effect, i.e. larger parental investment per offspring and/or hormonal regulation. Maternal effects are frequently observed for migration and other life-history traits (Dingle 1991) e.g. growth and reproductive potential (Rossiter 1991). These often 'set the stage' for a lifelong reaction to stressors by modifying the expression of genes in offspring that regulate behavioural, physiological and endocrinological responses to stressors (Mousseau & Fox 1998; Meaney 2001). Seasonality and availability of reliable cues, such as in the inundation forests, seem to favour strategies of non-genetic phenotypic variation (McGinley et al. 1987; Moran 1992). Stress-induced effects and stress-resistance strategies often persist for several generations, and such 'carry-over-effects' are suggested to be adaptive mechanisms on an ecological timescale, filling the gap between short-term individual and long-term evolutionary adaptation (Jablonka et al. 1995). According to Jablonka et al. (1995), these mechanisms might underlie the frequently observed rapid adaptation in invading populations e.g. in behaviour and life history (cf. Mooney & Cleland 2001; Reznick & Ghalambor 2001; Hänfling & Kollmann 2002). The resulting adaptive flexibility in phenotype is clearly an adaptive advantage for a species such as *P. obliterata* that colonises different biotopes.

4 GENETIC VARIATON

4.1 Material and Methods

4.1.1 Sample Collection

The sampling scheme was designed to assess allozyme variability both within and between populations of *P. obliterata* in different biotopes. Individuals were sampled from ten locations situated in the vicinity of Manaus, Amazonia State, Brazil (Fig. 50), and one locality approximately 1,000 km further downstream the Amazon River at Macapá; Amapá State, Brazil (Table 17). Two sample sites, Embrapa (EB) and Amapá (AM), were upland plantations, whereas the others were placed in inundation forests along the rivers Negro and Solimões, the sample sites thereby representing four different biotope types (Table 17). Given that one of the selected upland locations (AM) was geographically very remote, an additional site near Manaus would have been advantageous for the estimation of the respective intra-biotope variability. During sampling, however, *P. obliterata* could not be found in the local upland forests, i.e. the 'Capoeira' at the river Tarumã Mirím (Chapter 3.1.1.2) or the rainforest at the 'Reserva A. Ducke' (2°56' S, 59°58' W; Chapter 3.1.1.4.2), where the species is known to be rare (Golovatch, pers. commun.). The mixedwater area is territorially restricted, thus offering only one site for collections. Since *P. obliterata* is possibly dispersed by river (Chapter 1.2.2), four sample sites were chosen along the rivers Negro and Solimões, i.e. within the black- and whitewater inundation forests. Three of the selected sites were situated upstream of Manaus (Fig. 50), whereas the fourth site was located further downstream. With regard to analyses within one biotope type, samples from different sites are referred to as subpopulations. When comparing specimens from different biotopes, I will refer to populations. Most of the locations were sampled once (PG, AV, PQ, AM) or twice (JC, IP, IC) in the year 2003, while at four sites (MA, TM, LJ, EB) monthly collections were conducted in 2002/2003, but all data presented were pooled for each location. Each 30 to 50 random individuals, mainly adults or subadults, were taken. The date of sampling as well as the age and sex of the respective specimens were recorded. They were frozen alive and stored in a freezer at -70 °C until processed. To enable supplementary taxonomic investigations, a collection of *P. obliterata* from the different sites was preserved in alcohol.

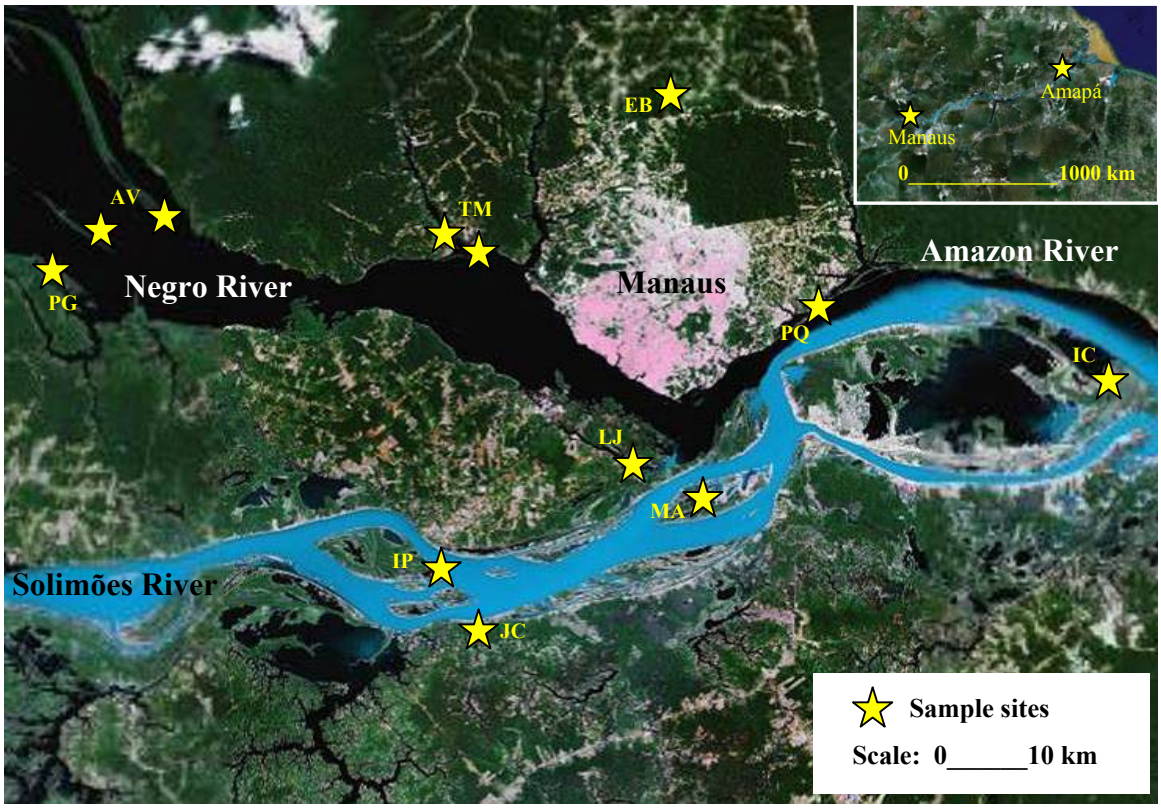

Figure 50. Sample sites of *P. obliterata* in the vicinity of Manaus, AM, Brazil. The inset map indicates the position of Amapá State where additional specimens were sampled. (Lake Janauacá: JC, Paciência Island: IP, Marchantaria Island: MA, Careiro Island: IC, Praia Grande: PG, Anavilhanas Islands: AV, Tarumã Mirím River: TM, Puraquequara River: PQ, Lake Janauarí: LJ, Embrapa: EB).

Table 17. Sample sites of *Poratia obliterata* subpopulations tested for enzyme variability.

Location	**Abbr.**	**Biotope type**	**N° of sub sites** (used for sampling)	**GPS-data** (study area centre)
Lake Janauacá	JC	Várzea	11	03°22.16 S 060°09.38 W
Paciência Island	IP	Várzea	8	03°18.61 S 060°11.55 W
Marchantaria Island	MA	Várzea	19	03°14.83 S 059°57.32 W
Careiro Island	IC	Várzea	9	03°08.76 S 059°36.16 W
Praia Grande	PG	Igapó	9	03°02.65 S 060°31.90 W
Anavilhanas Islands	AV	Igapó		
A			4	A: 03°00.68 S 060°29.33 W
B			2	B: 02°59.98 S 060°26.29 W
Tarumã Mirím River	TM	Igapó		
A			8	A: 03°01.03 S 060°10.98 W
B			7	B: 03°01.69 S 060°09.56 W
Puraquequara River	PQ	Igapó	6	03°04.80 S 059°51.50 W
Lake Janauarí	LJ	Várzea & Igapó (mixedwater)	15	03°13.12 S 060°01.18 W
Embrapa	EB	Terra firme	20	02°53.62 S 059°59.35 W
Amapá	AM	Terra firme	4	00°38.20 N 051°18.54 W

4.1.2 Agarose Gel Isoelectric Focussing (AGIF)

4.1.2.1 Sample Preparation

A minimum number of 30 adult or subadult individuals, with the exception of a few juveniles of the 6th developmental stage, per location were genotyped at six enzyme loci (enzyme commission classification numbers, EC, and abbreviations of the loci are listed in Table 18). This number yielded sufficient accuracy on the allele frequencies of the subpopulations. However, some specimens did not provide staining results for some of the loci. Therefore, sample sizes of a few data sets were reduced (see *N* in Table 24).

Table 18. Enzymes studied for genetic variability. The enzyme commission classification number (EC) and the abbreviations of loci are listed. Abbreviation: s, soluble isozyme.

Enzyme	EC	Locus
Acid Phosphatase	3.1.3.2	ACP
Glutamate-oxalacetate Transaminase (s)	2.6.1.1	GOT
Malic Enzyme (s)	1.1.1.40	ME
Glucose-phosphate Isomerase	5.3.1.9	PGI
Phophoglucomutase	2.7.5.1	PGM
Pyruvate Kinase	2.7.1.40	PK

Single individuals were placed in an Eppendorf vial filled with 100 µl homogenisation buffer (Table 19) and 20 µl DTT (Table 20) and frozen at -20 °C. For juveniles of the 6th stage only 80 µl of the mixture were applied. Subsequently, the sample was defrosted at ambient temperature and homogenised by ultrasonic disintegration (Table 21). Then the homogenate was frozen for at least two hours at -20 °C. It was finally defrosted at ambient temperature and centrifuged for 5 minutes at 10 000 rpm prior to the application upon gel. The remaining quantity was kept in cold storage at -20 °C.

Table 19. Staining solutions and homogenisation buffer

Description	pH	Chemical composition
Homogenisation buffer	7.4	10 ml of 0.12 M Tris, 0.1 M Histidine-HCl buffer: 1.5 g Tris(hydroxymethyl)-aminomethane (Serva) 2.2 g L-Histidine-HCl (Serva) ad 100 ml A. dest. 1 g sucrose (Serva) 4 mg EDTA-Na_2 (Tritiplex III) (Merck)
ACP staining solution	4.6	6 ml of 1 M Na acetate: 8.2 g Sodium acetate (Sigma) 10 ml Acetic acid [> 99 %] (Merck) ad 100 ml A. dest. 30 mg 1-Naphthyl phosphate (Sigma) in 1 ml A. dest. 50 mg Fast Blue BB (Sigma) in 1 ml H_2O (vortexed)
GOT staining solution	8.0	8 ml of Tris-Aspartic acid buffer: 6 g Tris(hydroxymethyl)-aminomethane (Serva) 2 g L-Aspartic acid (Sigma) ad 100 ml A. dest. 10 mg NADH (Sigma) 90 mg α-Ketoglutaric acid (Sigma) 5 mg Pyridoxal 5´-phosphate (Sigma) 5 mg MTT (Merck & Sigma) 35 µl Malate Dehydrogenase (Sigma)
ME staining solution	-	8 ml of Tris-Malic acid buffer: 6 g Tris(hydroxymethyl)-aminomethane (Serva) 3 g Malic acid (Serva) ad 100 ml A. dest. 40 mg $MgCl_2$*$6H_2O$ (Serva) 10 mg MTT (Merck & Sigma) 25 mg NADP (Sigma) 100 µl Meldola´s blue (2mg/ml A. dest) (Sigma)
PGI staining solution	7.6	8 ml of 0.2 M Tris, 0.19 M Histidine-HCl buffer: 2.4 g Tris(hydroxymethyl)-aminomethane (Serva) 4 g L-Histidine-HCl (Serva) 0.2 g Imidazole (Sigma) 0.1 g $MgCl_2$*$6H_2O$ (Serva) ad 100 ml A. dest.

Description	pH	Chemical composition
PGI staining solution	7.6	6 mg D-Fructose-6-phosphate (Sigma) 10 mg MTT (Merck & Sigma) 15 mg NAD (Sigma) 35 µl Glucose-6-phosphate Dehydrogenase from *Leuconostoc mesenteroides* (Sigma) 100 µl Meldola´s blue (2mg/ml A. dest) (Sigma)
PGM staining solution	8.0	8 ml of 0.5 M Tris-HCl buffer: 6 g Tris(hydroxymethyl)-aminomethane (Serva) 25 ml 1 N HCl (Merck) ad 100 ml A. dest. 15 mg α-D-Glucose-1-phosphate (Serva) 2 mg α-D-Glucose-1,6-bis-phosphate (Sigma) 10 mg $MgCl_2$*$6H_2O$ (Serva) 10 mg MTT (Merck & Sigma) 15 mg NAD (Sigma) 50 µl Glucose-6-phosphate Dehydrogenase from *Leuconostoc mesenteroides* (Sigma) 100 µl Meldola´s blue (2mg/ml A. dest) (Sigma)
PK staining solution	7.8	8 ml of 0.3 M Tris, 0.23 M Histidine-HCl buffer: 3.64 g Tris(hydroxymethyl)-aminomethan (Serva) 4.8 g L-Histidine-HCl (Serva) 0.74 g KCl (Merck) 1 mg $MgCl_2$*$6H_2O$ (Serva) ad 100 ml A. dest. 15 mg Adenosine 5´diphosphate (Sigma) 25 mg Phosphoenolpyrovate (Sigma) 11 mg NADH (Sigma) 10 mg MTT (Merck & Sigma) 35 µl L-Lactate Dehydrogenase (Sigma)

Table 20. Chemicals and consumables

Product description	Prepared solution
Dithiothreitol (DTT) (Sigma)	1 mg DTT/ml A. dest.
PlusOne Repel-Silane ES (Amersham biosciences)	
GelBond film (agarose) 124 x 258 mm (Amersham biosciences)	
Gel-Fix® for agarose 125 x 258 mm (Serva)	
Agarose IEF (Amersham)	
Ampholytes, FlukaBrand, IEF carrier ampholytes	
Ampholyte solutions pH 3.0-10.0, 4.0-6.0, 5.0-7.0, 5.0-8.0, 40 % in water, for electrophoresis (Sigma)	
Electrode strips (Amersham)	
Filter paper 0857 (Schleicher & Schuell Ltd.)	
Phenazine Methosulfate (PMS) (Sigma)	1 g PMS/20 ml A. dest.

Table 21. Laboratory equipment

Product description
Ultrasonic disintegrator 50 W (Neo Lab)
Glass plate, 260 x 125 x 3 mm (Amersham biosciences) Glass plate, 260 x 125 x 2 mm, U-frame (Amersham biosciences)
Multiphor™ II (flatbed) Electrophoresis System (Amersham biosciences)
Application strip silicone, 54 holes per 10 µl (Desaga Ltd.)

4.1.2.2 Preparation of the Agarose Gel

First the casting cassette had to be assembled (Tables 20 & 21). Following the instruction manual, the inner surface of the spacer plate (glass plate with a U-frame; Table 21) was coated with Repel-Silane (Table 20). Its hydrophobic side down, a GelBond film (Table 20) was placed onto the moistened glass plate without U-frame (Table 21). Using a roller, the foil was attached evenly to the plate, simultaneously removing bubbles and excessive water. The spacer plate was placed on the glass plate and the cassette was clamped together. It was warmed for 10 minutes in a heating cabinet at 70 °C. In the meantime 2 g sucrose, 170 mg agarose IEF (Table 20) and 15 ml de-ionised water were added into an Erlenmeyer flask (100 ml). The mixture was boiled for 1 minute in a microwave oven until the agarose dissolved completely. The process had to be disrupted every 15 to 20 seconds to agitate the flask and prevent the

liquid from boiling over. After the solution had cooled to 60 to 70 °C, the carrier ampholytes (Tables 20 & 22) were added rapidly. The liquid gel was filled up to 17 ml by heated de-ionised water and subsequently injected between the spacer plate and GelBond film. The cassette was left to stand for 15 minutes at ambient temperature and then placed in a refrigerator at 1 °C. After 1 hour, the gel (125x260x0.5 mm) could be removed from the cassette. It was stored in a humidity chamber overnight in the refrigerator and could be kept for up to one week. The thus prepared agarose electrofocussing gel of 1 % (w/v) molten agarose IEF contained 11.8 % (w/v) sucrose and 7.6 % (v/v) carrier ampholytes.

Table 22. Composition of carrier ampholytes for gels with wide and basic pH gradients. The pH range of the ampholyte solutions in which the enzymes were analysed is listed.

pH gradient	**pH range of the ampholyte solution**				**Enzyme**
	3.0-10.0	4.0-6.0	5.0-7.0	5.0-8.0	
wide	500 µl	400 µl	-	400 µl	ME, PGI, PK, ACP
basic	500 µl	-	400 µl	400 µl	PGM
basic	300 µl	-	1000 µl	-	GOT

4.1.2.3 Isoelectric Focussing

The gel was placed onto a moistened cooling plate of the Multiphor™ electrophoresis chamber (Table 21) at 12 °C, using approximately 1 ml water. The surface of the gel had to be dried carefully by applying moist filter paper. The anolyte solution was 0.01 M H_2SO_4, or 0.1 M H_3PO_4 (GOT); the catholyte solution was 1 M NaOH. Each 2 ml of the electrolyte solution were applied per electrode strip. For a sample application, up to 33 squares (5x5 mm) of filter paper (Table 20) were positioned in line at a certain distance to either anode or cathode (Table 23). In case of the PK the samples were applied using an application strip (Table 21).

Depending on the particular enzyme system, 7 to 10 µl (Table 23) of the supernatant from each sample vial were applied per paper square. To estimate displacements of the pH gradient at the edges of the gel, the first sample was applied once again in the end.

Maximum settings of the power unit were: V = 1500, mA = 50 and W = 10. After starting the run the power was raised until the voltage became 500 V. The run was finished after 45 to 60 minutes when a voltage of 1250 V was reached.

Table 23. Volume of sample solution and application area for six enzymes.

Enzyme	Volume (µl)	Application area
ACP	7	2 cm apart from cathode
GOT	9	2 cm apart from cathode
ME	7	2 cm apart from cathode
PGI	7	1 cm apart from anode
PGM	9	1 cm apart from cathode
PK	7	2 cm apart from cathode

4.1.2.4 Staining Procedure

Directly after separation, the gel was stained to visualise the allocation of the proteins, i.e. the enzyme reaction was combined with the formation of a dye, in most cases Formazan. A piece of filter paper was soaked with 6 to 8 ml of the freshly prepared staining solution (Table 19). After excess liquid was left to drip off, the staining paper was applied to the gel surface. The staining solution was left to act in an opaque case at ambient temperature until the bands were distinctly visible.

In case of PK and GOT, the formation of bands was monitored under long wave UV light and visualised by immersion in a PMS solution (Table 20).

The stained gel was placed between two overhead foils on a flatbed scanner. Figures of the gel with and without paper overlay as well as the staining paper itself were taken and saved on disk.

The gel was watered overnight and then covered with a wet filter paper and left to dry in a heating cabinet until the paper peeled off. The dried gel and staining paper were deposited in clear plastic binders, stored in a folder and kept in a dark and dry place.

4.1.2.5 Interpretation of Zymograms

The staining patterns represented the phenotypic variation of a particular enzyme encoded by different alleles at the respective locus. The electromorph configuration of different enzymes generally demonstrated that *Poratia obliterata* is a diploid organism. Monomeric enzymes (PGM, PK, and ACP) are shown phenotypically with either single or double bands. Heterozygotes for dimeric enzymes (PGI, GOT) show triplet patterns, while heterozygotes for the tetrameric ME exhibit five bands.

Single allozyme bands were assumed to correspond to unique alleles, but with the following caveat: individuals sharing the same allozyme phenotype may possess amino acid substitutions that do not result in detectable mobility differences. Hence the here defined 'electrophoretic' genotypes in fact may contain increased genetic variation, i.e. hidden polymorphism (Mitchell et al. 2004; Sperlich 1988).

Gene products with increasingly acidic IEPs (isoelectric points) were termed by even numbers, while those with more basic IEPs were designated by means of odd numbers. Consequently, genotypes were coded with two-digit numbers, with different numbers representing heterozygotes according to the varied mobility of their gene products, but equal numbers denoting homozygotes.

4.1.3 Data Analysis and Statistics

The GENEPOP software package, updated version 3.4 (Raymond & Rousset 1995) was used to estimate allele frequencies and average heterozygosity (H_{obs} and H_{exp}; the number of observed and expected heterozygotes, respectively, were computed using Levene's correction), and also to test for deviations of genotype distributions from Hardy-Weinberg equilibrium as well as genotypic linkage disequilibrium between pairs of loci. The effective number of alleles was manually calculated according to $k_e = 1/(1-H)$ (Sperlich 1988). The significance of multiple, pairwise comparisons of both k_e and k/k_e in the different biotopes was tested by the non-parametrical Kruskal-Wallis-test (Sachs 1999) using the software SAS, version 9.1. (SAS Institute Inc. 2003).

In addition, analogous probability tests were performed for allelic and genotypic differentiation between pairs of populations as well as subpopulations. Single locus and multilocus estimates of pairwise F_{ST} as short-term genetic distances between subpopulations were computed by a 'weighted analysis' of variance (Weir & Cockerham 1984). Multilocus F_{IS} for all subpopulations were estimated and the probability that F-values over all subpopulations unequalled zero was calculated by the FSTAT program (Goudet 1995). The F_{ST} values were used to estimate the level of gene flow (M) in the test for genetic isolation by geographic distance (Slatkin 1993) performed in GENEPOP. Linear geographic distances between pairs of subpopulations were calculated by the DIST program (www.fcc.gov) using GPS-data of sample sites centres (Table 17: average values for AV and TM). The effective number of migrants

(*Nm*) exchanged between local subpopulations in a subdivided population was calculated according to the private allele method (Slatkin 1985).

As GENEPOP performs tests for large sample sizes solely by means of the Markov chain approximation, the Fisher-exact test to compare the genotypic structure of the subpopulations was carried out using the software SAS (SAS Institute Inc. 2003).

Using the Arlequin program, version 2.000 (Schneider et al. 2000), the average pairwise differences among and within the study subpopulations were computed based on conventional *F*-statistics. Mantel tests were performed under 1000 random permutations. To evaluate the population genetic structure, a locus-by-locus hierarchical analysis of molecular variance (AMOVA) based on allele frequencies was performed. The effects of biotope type, ecological condition and geographic position were analysed. The probability of having more extreme variance components than the observed values by chance alone was tested under 5000 random permutations of populations across groups, individuals across populations but within the same group, and individuals across populations without regard to either their original population or group.

For all statistical tests a significance level of 5 % was accepted. The sequential Bonferroni procedure was applied when multiple tests were done in order to compensate for an inflating effect of the type I error *P* (Rice 1989; Jaccard & Wan 1996).

Genetic distances based on allelic (Nei 1978) and genotypic frequencies (Tomiuk & Loeschcke 1991) between pairs of subpopulations were estimated using the POPDIST program, version 1.1.1 (Guldbrandtsen et al. 2000), with the standard errors being calculated by means of the Jacknife procedure. Dendrograms on the basis of both Nei's and Tomiuk & Loeschcke's calculated genetic distances were constructed by the neighbour-joining method (Saitou & Nei 1987) using the MEGA2 software package (Kumar et al. 2001) and were subsequently revised in Microsoft PowerPoint.

4.2 Results

4.2.1 Zymograms

Details of selected enzymograms of the enzyme systems PGI, PGM, ME, GOT, PK and ACP are shown in Figure 51.

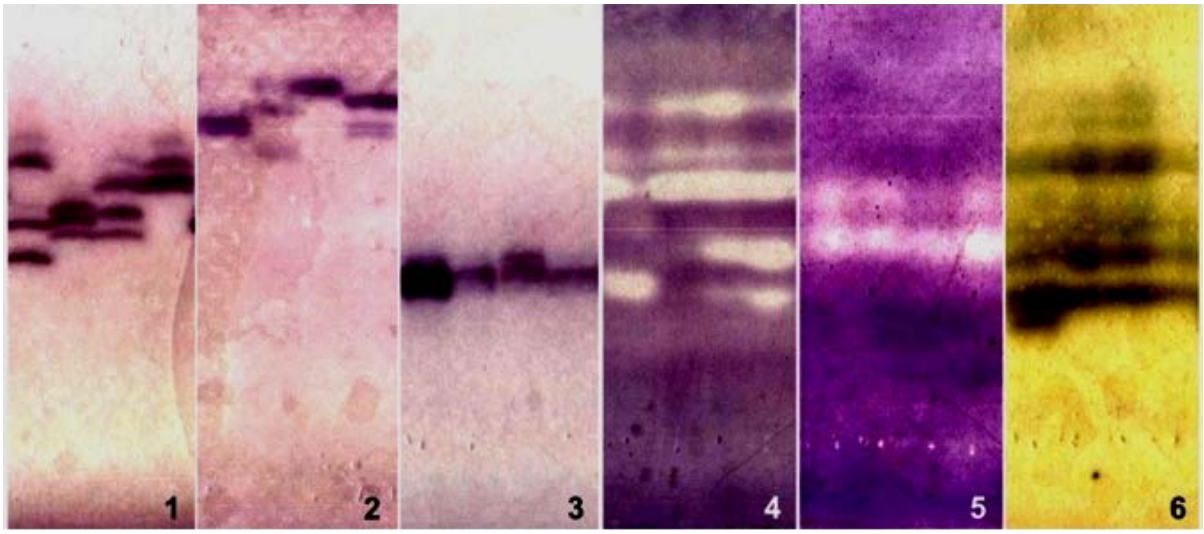

Figure 51. Details of selected zymograms of the PGI, PGM, ME, GOT, PK and ACP systems. Scan of gel & staining paper overlay.
N° 1: zymogram of PGI, phenotypes: 9-3, 6-2, 8-2, 9-8.
N° 2: zymogram of PGM, phenotypes: 4-4, 5-3, 1-1, 2-2.
N° 3: zymogram of ME, phenotypes: 5-1, 1-1, 2-1, 1-1.
N° 4: zymogram of GOT, phenotypes: 3-3, 1-1, 3-1, 3-1.
N° 5: zymogram of PK, phenotypes: 2-1, 2-1, 1-1, 1-1.
N° 6: zymogram of ACP, phenotypes: 3-1, 2-1, 2-1, 1-1.

All six loci were polymorphic accepting the 95 % criterion, i.e. the frequency of the most common allele was lower than 0.95 (Sperlich 1988).

PGI shows up to eight distinct gene products with single bands in the homozygous and three banded patterns in the heterozygous state.

PGM displays six different electrophoretic positions; homozygous individuals have one band, heterozygotes a double banded pattern.

ME is revealed as up to four distinct gene products. Since it has been described as a tetrameric enzyme (Harris & Hopkinson 1976), ‘broad’ bands were interpreted as heterozygotes, ‘small’ bands as homozygotes.

GOT exhibits three different electrophoretic positions, the more common genotypes GOT 1-1, GOT 3-1 and GOT 3-3 are shown.

PK and ACP show two and four distinct gene products, respectively. Heterozygotes are displayed as two bands with equal intensity.

4.2.2 Allelic Variation within and among Biotope Types

The allele frequencies for the six enzyme loci in each subpopulation are presented in Table 24. Some remarks on the most frequent alleles and population specific differences are given hereafter.

PGI: Alleles 4, 6 and 8 were the most common across all biotope types, their combined frequency ranging from 0.416 to 0.783 within the subpopulations. However, additional alleles occurred relatively frequent in individual subpopulations of different biotope types; e.g. allele 1 with a frequency of 0.167 in MA (Várzea) and LJ (mixedwater); allele 2 with a frequency of 0.150 and 0.167, respectively, in JC (Várzea) and AM (Terra firme); allele 5 with a frequency of 0.150 in JC (Várzea); and allele 9 with a frequency of 0.150 and 0.233, respectively, in JC (Várzea) and TM (Igapó). Allele 3 was less frequent (0.033 to 0.133) in all biotopes and absent from two (AV, TM) of the four Igapó subpopulations.

PGM: With the exception of the subpopulations AV (Igapó) and AM (Terra firme), allele 3 was the most common, its frequency ranging from 0.444 to 0.790 in the remaining nine subpopulations. Allele 2 was most frequent in AV (0.565) and AM (0.806), but also revealed substantial frequencies (0.156-0.359) in the other subpopulations. Allele 1 was absent on Terra firme but occurred in low frequencies (0.011-0.163) in six of the nine inundation forest (Várzea, Igapó, mixedwater) subpopulations. Allele 6 was private to the subpopulation PG (Igapó).

ME: Four alleles were recorded at the ME locus, but allele 1 was the most frequent in all populations, with its frequency ranging from 0.750 to 0.967. Alleles 2 and 3 showed no consistent frequency across biotope types, either of them being the second common allele with a frequency of 0.067 to 0.145.

GOT: Allele 1 was the most frequent allele at the GOT locus for all populations and ranged from 0.595 to 1.000. All individuals of the subpopulation AM (Terra firme) were homozygous GOT 1-1. With the exception of the subpopulation IP (Várzea), allele 3 was the second common one, its frequency ranging from 0.033 up to 0.405. Allele 2 was rather frequent (0.233) in the subpopulation IP

(Várzea), but only occurred (0.167) in two other subpopulations (PG and AV; Igapó).

PK: High frequencies up to 0.806 were found for either of both alleles 1 and 2, but no consistent patterns of allele frequency distribution across subpopulations within or among biotope types were revealed.

ACP: Four alleles were recorded at the ACP locus. Allele 1 was most frequent in all populations and ranged from 0.552 to 0.83. Allele 2 was the second common allele (0.117-0.310) in all biotopes. Whereas allele 3 was relatively frequent (0.138) in the subpopulation AM (Terra firme), it was only found in low frequencies (0.017-0.059) in some subpopulations of the inundation forests (Várzea, Igapó and mixedwater). Allele 4 was private to the subpopulation IP (Várzea).

Summarising, no consistent patterns of allelic variation of subpopulations within and among biotope types could be observed. The mean number of alleles per locus (*A*) ranged from 3 to 4 and was similar across all eleven subpopulations and four biotope types (Table 24).

Table 24. Allele frequencies at six enzyme loci for all eleven subpopulations grouped into four biotope types. The mean number of alleles per locus (*A*); expected and observed level of heterozygosity (H_{exp}, H_{obs}) and number of specimens analysed (*N*) are given. Abbreviations: MW, mixedwater; TF, Terra firme.

Locus	**Allele**	**Várzea**				**Igapó**				**MW**	**TF**	
		JC	**IP**	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
PGI	*N*	30	30	30	30	30	29	30	30	30	30	30
	1	0.000	0.050	0.167	0.017	0.083	0.172	0.067	0.083	0.167	0.033	0.050
	2	0.150	0.133	0.133	0.083	0.100	0.155	0.067	0.167	0.067	0.117	0.167
	3	0.133	0.033	0.067	0.017	0.017	0.000	0.000	0.050	0.067	0.017	0.100
	4	0.083	0.350	0.167	0.117	0.167	0.379	0.117	0.183	0.033	0.150	0.300
	5	0.150	0.017	0.000	0.000	0.000	0.000	0.083	0.000	0.050	0.000	0.017
	6	0.000	0.267	0.117	0.483	0.300	0.241	0.233	0.350	0.400	0.317	0.067
	8	0.333	0.033	0.217	0.183	0.233	0.034	0.200	0.133	0.100	0.300	0.150
	9	0.150	0.117	0.133	0.100	0.100	0.017	0.233	0.033	0.117	0.067	0.150
H_{exp}		0.810	0.783	0.858	0.714	0.814	0.756	0.836	0.801	0.789	0.781	0.834
H_{obs}		0.700	0.733	0.633	0.367	0.733	0.448	0.667	0.767	0.733	0.467	0.700
PGM	*N*	31	32	47	30	27	31	46	30	38	46	31
	1	0.000	0.016	0.011	0.000	0.019	0.081	0.163	0.000	0.079	0.000	0.000
	2	0.177	0.157	0.160	0.217	0.296	0.565	0.217	0.200	0.224	0.359	0.806
	3	0.790	0.641	0.500	0.667	0.444	0.161	0.500	0.717	0.539	0.554	0.194
	4	0.032	0.172	0.298	0.067	0.130	0.177	0.120	0.083	0.158	0.054	0.000
	5	0.000	0.016	0.032	0.050	0.037	0.016	0.000	0.000	0.000	0.033	0.000
	6	0.000	0.000	0.000	0.000	0.074	0.000	0.000	0.000	0.000	0.000	0.000
H_{exp}		0.349	0.544	0.642	0.510	0.704	0.627	0.669	0.447	0.636	0.566	0.317
H_{obs}		0.226	0.469	0.447	0.300	0.407	0.355	0.522	0.200	0.526	0.413	0.194

Locus	Allele	Várzea				Igapó				MW	TF	
		JC	IP	MA	IC	PG	AV	TM	PQ	LJ	EB	AM
ME	*N*	29	29	34	30	30	30	29	26	29	33	31
	1	0.897	0.931	0.750	0.933	0.883	0.967	0.810	0.923	0.828	0.818	0.806
	2	0.103	0.000	0.000	0.067	0.100	0.017	0.000	0.000	0.000	0.015	0.000
	3	0.000	0.069	0.191	0.000	0.017	0.017	0.138	0.077	0.103	0.106	0.145
	5	0.000	0.000	0.059	0.000	0.000	0.000	0.052	0.000	0.069	0.061	0.048
H_{exp}		0.189	0.131	0.403	0.127	0.213	0.066	0.327	0.145	0.305	0.320	0.332
H_{obs}		0.138	0.138	0.500	0.067	0.233	0.067	0.379	0.154	0.310	0.364	0.355
GOT	*N*	21	30	34	27	21	30	30	30	30	31	30
	1	0.833	0.717	0.882	0.852	0.595	0.867	0.967	0.900	0.700	0.871	1.000
	2	0.000	0.233	0.000	0.000	0.000	0.017	0.000	0.017	0.000	0.000	0.000
	3	0.167	0.050	0.118	0.148	0.405	0.117	0.033	0.083	0.300	0.129	0.000
H_{exp}		0.285	0.437	0.211	0.257	0.494	0.239	0.066	0.186	0.427	0.228	0.000
H_{obs}		0.238	0.167	0.235	0.222	0.619	0.133	0.067	0.200	0.333	0.258	0.000
PK	*N*	30	31	27	30	30	29	28	31	29	33	29
	1	0.800	0.565	0.352	0.567	0.383	0.724	0.321	0.806	0.569	0.394	0.328
	2	0.200	0.435	0.648	0.433	0.617	0.276	0.679	0.194	0.431	0.606	0.672
H_{exp}		0.325	0.500	0.465	0.499	0.481	0.407	0.444	0.317	0.499	0.485	0.448
H_{obs}		0.333	0.484	0.482	0.533	0.367	0.414	0.571	0.323	0.586	0.667	0.517
ACP	*N*	30	29	38	29	28	26	34	29	34	38	29
	1	0.833	0.690	0.816	0.707	0.821	0.808	0.794	0.828	0.750	0.776	0.552
	2	0.117	0.276	0.158	0.276	0.161	0.192	0.206	0.172	0.191	0.224	0.310
	3	0.050	0.017	0.026	0.017	0.018	0.000	0.000	0.000	0.059	0.000	0.138
	4	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H_{exp}		0.295	0.456	0.313	0.431	0.305	0.317	0.332	0.290	0.403	0.352	0.590
H_{obs}		0.267	0.379	0.316	0.586	0.357	0.385	0.353	0.345	0.412	0.447	0.621
All loci												
A		3.00	4.00	3.67	3.33	3.83	3.50	3.50	3.17	3.67	3.67	3.16
H_{exp}		0.375	0.475	0.482	0.423	0.502	0.402	0.446	0.364	0.510	0.455	0.420
H_{obs}		0.317	0.395	0.435	0.346	0.453	0.300	0.426	0.331	0.484	0.436	0.398

4.2.3 Heterozygosis and Hardy-Weinberg Distribution

The mean degree of observed heterozygosity (H_{obs}) noted in the eleven subpopulations of *P. obliterata* ranged from 0.300 to 0.484, while the mean expected degree of heterozygosity (H_{exp}) ranged from 0.364 to 0.510 (Table 24). Whereas the lowest mean level of heterozygosity was observed in Várzea (0.378) and Igapó (0.373) populations, the Terra firme (0.417) and particularly the mixedwater inundation forest population (0.484) comprised higher proportions of heterozygotes.

Interestingly, 40 % of all comparisons across six loci showed distinct deviations among the observed and expected number of heterozygotes in the eleven subpopulations, more

precisely, 29 % of the comparisons exhibited heterozygous deficiencies, while 11 % revealed an excess of heterozygotes (predominantly at the PK locus).

However, when testing for Hardy-Weinberg proportions, the genotype distributions of only five cases (PGI locus: IC and EB; PGM locus: PG and AV; GOT locus: IP; Table 25) deviated significantly from the Hardy-Weinberg equilibrium, accepting a 5 % significance level (applying sequential Bonferroni procedure), suggesting a significant lack of heterozygotes in the respective subpopulations.

Table 25. Tests of Hardy-Weinberg equilibrium in eleven subpopulations of *P. obliterata* for six enzyme loci. Bold numbers consider significant deviations from Hardy-Weinberg distribution accepting a 5 % significance level (applying sequential Bonferroni procedure).

Loci	**Subpopulation**										
	JC	IP	MA	IC	PG	AV	TM	PQ	LJ	EB	AM
PGI	0.109	0.328	0.007	**0.000**	0.478	0.002	0.026	0.594	0.182	**0.000**	0.013
PGM	0.076	0.779	0.012	0.009	**0.000**	**0.000**	0.034	0.001	0.111	0.134	0.054
ME	0.249	1.000	0.381	0.101	1.000	1.000	1.000	1.000	0.607	1.000	0.497
GOT	0.448	**0.000**	1.000	0.454	0.367	0.058	1.000	1.000	0.378	1.000	-
PK	1.000	1.000	1.000	1.000	0.252	1.000	0.196	1.000	0.453	0.035	0.671
ACP	0.592	0.018	1.000	0.085	1.000	0.545	1.000	0.558	1.000	0.158	0.704

4.2.4 Effective Number of Alleles per Locus

The effective number of alleles (k_e) indicates the number of alleles that can be found at a given level of heterozygosity assuming all alleles have the same frequency (Sperlich 1988), i.e. it measures the evenness of allelic frequencies (El Mousadik & Petit 1996). Estimates of k_e per locus in the different biotope types are listed in Table 26. Considering all loci, the population from the mixedwater inundation forest had a greater number of effective alleles (2.1) than the populations in the other biotope types (Várzea, 1.8; Igapó, 1.9; Terra firme, 2.0). However, the differences across all loci and populations were not significant (*H*-Test of Kruskal and Wallis: $\chi^2 = 3.596$, $df = 3$, $P = 0.309$).

The ratio of actual (k) to effective (k_e) number of alleles per locus estimates the genetic variability within a population (Sperlich 1988). Values of k/k_e across all loci in the four biotope types are listed in Table 27. Allelic variation within the Várzea population was relatively high (1.9) compared to the remaining three populations (Igapó, 1.8;

mixedwater, 1.7; Terra firme, 1.7). Again, no significant differences across all loci and populations could be demonstrated statistically (*H*-Test of Kruskal and Wallis: $\chi^2 = 3.866$, $df = 3$, $P = 0.276$).

Table 26. Mean number of effective alleles (k_e) per locus in the four biotope types (estimated $k_e = 1/(1-H)$; Sperlich 1988). *n* indicates the number of subpopulations of *P. obliterata* analysed per biotope; numbers in brackets give the range of the total number of alleles observed in the subpopulations (Table 24).

Loci	**Várzea** (*n* = 4)	**Igapó** (*n* = 4)	**Mixedwater** (*n* = 1)	**Terra firme** (*n* = 2)
PGI	2.8 (6-8)	3.2 (6-7)	3.7 (8)	2.6 (7-8)
PGM	1.6 (3-5)	1.7 (3-6)	2.1 (4)	1.5 (2-4)
ME	1.4 (2-3)	1.3 (2-3)	1.4 (3)	1.6 (3-4)
GOT	1.3 (2-3)	1.6 (2-3)	1.5 (2)	1.2 (1-2)
PK	1.9 (2)	1.8 (2)	2.4 (2)	2.6 (2)
ACP	1.7 (3-4)	1.6 (2-3)	1.7 (3)	2.2 (2-3)
All loci	1.8 (2-8)	1.9 (2-7)	2.1 (2-8)	2.0 (1-8)

Table 27. Ratio of actual (*k*) and effective (k_e) number of alleles per locus for each biotope (for details see Sperlich, 1988). *n* indicates the number of subpopulations of *P. obliterata* analysed per biotope type.

Loci	**Várzea** (*n* = 4)	**Igapó** (*n* = 4)	**Mixedwater** (*n* = 1)	**Terra firme** (*n* = 2)
PGI	2.5	2.1	2.2	2.9
PGM	2.7	2.6	1.9	2
ME	1.6	1.9	2.1	2.2
GOT	1.8	1.6	1.3	1.3
PK	1.1	1.1	0.8	0.8
ACP	1.9	1.4	1.8	1.1
All loci	1.9	1.8	1.7	1.7

4.2.5 Genotypic Linkage Equilibrium between Loci

No significant genotypic association between pairs of loci at a significance level of 5 % could be detected; i.e. allelic segregation at the different loci is unrelated ($P > 0.01$, Bonferroni corrections; Table 28). Therefore, the loci provide independent information on population structure.

Table 28. Tests of genotypic linkage disequilibrium between pairs of six loci across all eleven subpopulations. The χ^2-values, the degree of freedom (*df*) and the probability of type I error are listed. No significant genotypic linkage found between loci at a 5 % significance level (applying sequential Bonferroni procedure).

Locus pair	χ^2	*df*	*P*-value
PGI & PGM	30.019	20	0.070
PGI & ME	13.714	22	0.911
PGM & ME	12.626	20	0.893
PGI & GOT	6.403	4	0.171
PGM & GOT	16.779	14	0.268
ME & GOT	7.077	4	0.132
PGI & PK	27.307	22	0.200
PGM & PK	17.850	20	0.597
ME & PK	23.134	22	0.394
GOT & PK	3.979	4	0.409
PGI & ACP	28.953	22	0.146
PGM & ACP	36.938	20	0.012
ME & ACP	22.029	22	0.458
GOT & ACP	8.482	4	0.075
PK & ACP	29.406	22	0.134

4.2.6 Genetic Differentiation among Populations

4.2.6.1 Within-Subpopulation Variance of Genetic Diversity

The inbreeding coefficient F_{IS} (Wright 1951) measures the reduction in heterozygosity of individuals due to non-random mating within their subpopulation (Hartl 1988). Estimates of F_{IS} per subpopulation over all loci are significant accepting a 5 % significance level (Table 29). The mean F_{IS}-value across all loci and subpopulations was 0.131 ± 0.067, indicating both heterozygote deficiency and non-random mating within subpopulations. Significant correlations of genes of different individuals in the same subpopulation over all locations were revealed for three loci (PGI, 0.210; PGM, 0.315; GOT, 0.144; $P < 0.01$). The extent of the consequently indicated coancestry was greater for the subpopulations of inundation forests (Várzea, 0.147 ± 0.059; Igapó, 0.146 ± 0.080; mixedwater, 0.158; Table 29) than for those living on Terra firme (0.053 ± 0.008).

Table 29. Estimate of F_{IS}-value per subpopulation of *P. obliterata* over all loci (PGI, PGM, ME, GOT, PK, ACP). F_{IS}-values are significant accepting a 5% significance level. Abbreviation: MW, mixedwater.

Subpopulation										
Biotope type										
Várzea				Igapó				MW	Terra firme	
JC	IP	MA	IC	PG	AV	TM	PQ	LJ	EB	AM
0.170	0.170	0.059	0.189	0.113	0.266	0.094	0.112	0.158	0.047	0.058

Single locus estimates of average pairwise differences within the eleven subpopulations were of similar magnitude than the average pairwise differences between them (values are based on conventional *F*-statistics, but for detailed results see Table 30a-f, appendix).

4.2.6.2 Genetic Variability between Subpopulations

The effects of population subdivision are measured by the fixation index F_{ST} (Wright 1951), which is the reduction in heterozygosity of a subpopulation due to random genetic drift (Hartl 1988). The F_{ST}-values over all subpopulations of *P. obliterata* were estimated by FSTAT (Goudet 1995) and are significant for all loci (PGI, 0.047; PGM, 0.116; ME, 0.033; GOT, 0.094; PK, 0.124; ACP, 0.017; $P < 0.02$), suggesting both allelic and genotypic differentiation among subpopulations. Estimates of pairwise F_{ST}-values between the subpopulations over all loci were obtained by GENEPOP (Raymond & Rousset 1995) and are listed in Table 31 (single locus F_{ST} were also estimated, detailed results are given in Table 32). The mean F_{ST}-value across all loci and subpopulations was 0.079 ± 0.052. The differentiation was less pronounced in the Várzea (0.066 ± 0.029) than in the other biotopes (Igapó, 0.092 ± 0.027; Terra firme, 0.092). Among inundation forest subpopulations (0.068 ± 0.039), the one from the mixedwater area was most similar to all others (LJ: 0.042 ± 0.026), whereas individual subpopulations in both Várzea and Igapó were genetically fairly distinct (JC: 0.096 ± 0.036; AV: 0.107 ± 0.025). Comparing Terra firme and inundation forests, the local subpopulation EB was genetically close (0.044 ± 0.034), but the geographically distant subpopulation AM was very different (0.154 ± 0.045) from the inundation forest subpopulations.

Table 31. Multilocus (PGI, PGM, ME, GOT, PK, ACP) estimates of pairwise F_{ST}-values among the studied subpopulations of *P. obliterata*. F_{ST}-values are significant accepting a 5% significance level.

	IP	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	0.082	0.106	0.075	0.113	0.158	0.119	0.040	0.076	0.096	0.236
IP		0.047	0.023	0.051	0.086	0.054	0.039	0.032	0.043	0.150
MA			0.060	0.034	0.114	0.011	0.092	0.035	0.023	0.127
IC				0.037	0.102	0.046	0.024	0.012	0.016	0.160
PG					0.094	0.042	0.102	0.016	0.016	0.124
AV						0.122	0.093	0.083	0.096	0.105
TM							0.101	0.039	0.012	0.112
PQ								0.041	0.070	0.216
LJ									0.025	0.152
EB										0.092

Table 32. Single locus estimates of pairwise F_{ST}-values among the studied subpopulations of *P. obliterata.* The results for six enzyme loci (PGI, PGM, ME, GOT, PK, ACP) are listed according to Table 35 for each sample site. F_{ST}-values are significant accepting a 5% significance level.

	Subpopulation									
	Habitat type									
	Várzea			**Igapó**				**Mixed-water**	**Terra firme**	
	IP	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	0.128	0.032	0.149	0.071	0.155	0.047	0.107	0.129	0.068	0.044
	0.028	0.115	0.001	0.108	0.359	0.086	-0.014	0.063	0.071	0.524
	0.031	0.094	-0.015	-0.017	0.029	0.057	0.030	0.042	0.038	0.059
	0.072	-0.010	-0.024	0.111	-0.020	0.084	0.005	0.023	-0.015	0.176
	0.105	0.332	0.104	0.291	-0.001	0.370	-0.016	0.103	0.282	0.361
	0.042	-0.011	0.043	-0.012	-0.002	0.003	-0.007	0.002	0.013	0.111
IP		0.042	0.060	0.030	-0.003	0.045	0.017	0.068	0.051	0.020
		0.013	-0.005	0.030	0.243	0.023	-0.005	-0.001	0.039	0.423
		0.074	0.015	0.027	0.007	0.032	-0.017	0.021	0.024	0.034
		0.103	0.083	0.157	0.077	0.171	0.096	0.100	0.095	0.221
		0.071	-0.016	0.046	0.038	0.099	0.113	-0.016	0.044	0.093
		0.023	-0.015	0.022	0.009	0.004	0.022	-0.002	-0.001	0.015
MA			0.076	0.007	0.045	0.010	0.028	0.050	0.021	0.004
			0.051	0.022	0.177	0.031	0.061	0.009	0.066	0.362
			0.114	0.079	0.133	-0.004	0.062	0.006	0.005	-0.006
			-0.013	0.197	-0.017	0.035	-0.010	0.082	-0.013	0.099
			0.073	-0.018	0.232	-0.013	0.342	0.076	-0.009	-0.015
			0.023	-0.014	-0.012	-0.008	-0.012	-0.005	-0.001	0.097
IC				0.007	0.092	0.035	0.006	0.011	0.006	0.110
				0.028	0.244	0.030	-0.019	0.010	0.012	0.403
				-0.007	-0.002	0.069	0.017	0.051	0.049	0.072
				0.141	-0.020	0.061	-0.003	0.043	-0.016	0.138
				0.048	0.037	0.102	0.112	-0.015	0.046	0.096
				0.023	0.011	0.005	0.023	-0.000	-0.000	0.024

	Subpopulation									
	Habitat type									
	Várzea			Igapó				Mixed-water	Terra firme	
	IP	MA	IC	PG	AV	TM	PQ	LJ	EB	AM
PG					0.044	0.002	-0.004	0.018	-0.016	0.037
					0.089	0.008	0.052	-0.001	0.003	0.244
					0.033	0.044	0.025	0.032	0.028	0.046
					0.172	0.359	0.231	0.004	0.175	0.444
					0.195	-0.009	0.301	0.050	-0.015	-0.011
					-0.013	-0.010	-0.014	-0.004	-0.002	0.096
AV						0.077	0.024	0.084	0.069	0.035
						0.145	0.284	0.157	0.143	0.074
						0.086	0.011	0.066	0.066	0.088
						0.033	-0.015	0.066	-0.018	0.097
						0.269	0.002	0.037	0.188	0.261
						-0.014	-0.013	-0.006	-0.009	0.083
TM							0.030	0.026	0.013	0.036
							0.044	-0.007	0.030	0.304
							0.023	-0.013	-0.012	-0.015
							0.013	0.211	0.045	0.017
							0.379	0.105	0.001	-0.014
							-0.011	-0.006	-0.011	0.077
PQ								0.015	0.003	0.047
								0.019	0.031	0.450
								0.014	0.017	-0.025
								0.110	-0.007	0.072
								0.111	0.291	0.371
								-0.001	-0.003	0.103
LJ									0.034	0.100
									0.016	0.324
									-0.014	-0.012
									0.067	0.285
									0.049	0.098
									-0.005	0.044
EB										0.053
										0.254
										-0.011
										0.115
										-0.002
										0.067

The results of Fisher exact tests to compare the genotypic structure of the eleven subpopulations are given in Table 33 and follow the same trend as the F_{ST}-values.

The genotypic distinction over all subpopulations revealed a rather heterogeneous pattern for all loci except the ACP locus, which did not provide any significant data

(P < 5 % significance level corrected by the sequential Bonferroni procedure). The mixedwater inundation forest subpopulation LJ hardly showed any genotypic differentiation to subpopulations of both Várzea and Igapó, since only three out of 48 comparisons (6 %) revealed significant differences between the respective subpopulations (JC: PGI; IP: GOT; AV: PGM). In contrast, genotypic discrimination among subpopulations within the Várzea (seven out of 36 comparisons revealed significant differences: 19 %) and Igapó (six out of 36 comparisons revealed significant differences: 17 %), as well as differentiation between the Várzea and Igapó subpopulations (14 out of 96 comparisons revealed significant differences: 15 %), were of almost threefold magnitude. The regional Terra firme subpopulation EB hardly showed any genotypic discrimination to subpopulations of inundation forests (five out of 54 comparisons revealed significant differences: 9 %; JC: PGI, PK; AV: PGM, PK; PQ: PK). In contrast, the geographically remote Terra firme subpopulation AM was genetically distinct to all remaining subpopulations, in particular those of inundation forests (16 out of 54 comparisons revealed significant differences: 30 %), whereas it was genetically closest to the other Terra firme subpopulation EB (one out of six comparisons revealed a significant difference at the PGM locus: 17 %).

Table 33. Fisher exact test of genotypic homogeneity between pairs of eleven subpopulations of *P. obliterata* sampled in four biotope types. Results per locus (PGI, PGM, ME, GOT, PK, ACP) were listed according to Table 35 for each sample site. Bold numbers consider significant differences between subpopulations accepting a 5 % significance level (applying sequential Bonferroni procedure).

	Subpopulation									
	Habitat type									
	Várzea			**Igapó**				**Mixed-water**	**Terra firme**	
	IP	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	**0.000**	0.036	**0.000**	0.002	**0.000**	0.002	**0.000**	**0.000**	**0.000**	0.006
	0.194	0.004	0.820	0.004	**0.000**	0.026	0.604	0.018	0.185	**0.000**
	0.013	**0.000**	0.707	0.731	0.190	**0.000**	0.013	0.001	0.002	**0.000**
	0.003	0.540	0.871	0.023	0.902	0.336	0.929	0.071	0.447	0.037
	0.030	**0.000**	0.027	**0.000**	0.616	**0.000**	1.000	0.016	**0.000**	**0.000**
	0.082	0.753	0.006	0.335	0.075	0.106	0.101	0.689	0.010	0.013
IP		0.055	0.011	0.242	0.099	0.007	0.245	0.005	0.003	0.036
		0.609	0.610	0.064	**0.000**	0.487	0.724	0.537	0.107	**0.000**
		0.000	0.047	0.012	0.271	0.061	1.000	0.221	0.104	0.138
		0.000	0.003	**0.000**	0.012	0.003	0.013	**0.000**	0.001	**0.000**
		0.086	1.000	0.123	0.179	0.010	0.025	0.828	0.025	0.028
		0.237	0.101	0.296	0.358	0.407	0.254	0.323	0.101	0.069

	Subpopulation									
	Habitat type									
	Várzea			Igapó				Mixed-water	Terra firme	
	IP	MA	IC	PG	AV	TM	PQ	LJ	EB	AM
MA			0.071	0.407	0.095	0.073	0.255	0.155	0.174	0.201
			0.083	0.002	**0.000**	0.008	0.058	0.128	0.002	**0.000**
			0.000	**0.000**	**0.000**	0.668	0.009	0.158	0.350	0.388
			0.759	0.039	0.897	0.086	0.542	0.044	1.000	0.005
			0.073	0.596	0.001	0.552	**0.000**	0.057	0.347	0.927
			0.056	0.947	0.554	0.686	0.635	0.840	0.118	0.014
IC				0.181	0.020	0.040	0.058	0.1738	0.923	0.003
				0.032	**0.000**	0.075	0.861	0.1299	0.621	**0.000**
				0.197	1.000	**0.000**	0.020	0.0017	0.001	0.001
				0.077	1.000	0.146	1.000	0.1464	0.762	0.011
				0.092	0.199	0.014	0.015	1.0000	0.044	0.027
				0.142	0.180	0.125	0.114	0.1914	0.379	0.129
PG					0.003	0.418	0.705	0.1496	0.443	0.101
					0.021	0.148	0.277	0.0319	0.145	**0.000**
					0.103	0.001	0.022	0.0033	0.005	0.001
					0.009	**0.000**	0.016	0.5659	0.068	**0.000**
					0.002	0.114	**0.000**	0.0482	0.045	0.290
					0.885	0.879	1.000	0.8494	0.305	0.028
AV						0.002	0.272	0.005	0.011	0.005
						0.000	**0.000**	**0.000**	**0.000**	0.048
						0.003	0.172	0.030	0.010	0.006
						0.345	0.894	0.054	0.112	0.023
						0.000	0.559	0.173	**0.000**	**0.000**
						1.000	0.786	0.439	0.797	0.018
TM							0.024	0.026	0.115	0.010
							0.372	0.433	0.015	**0.000**
							0.114	0.782	0.962	0.960
							0.254	0.001	0.081	0.492
							0.000	0.011	0.553	1.000
							1.000	0.369	0.545	0.007
PQ								0.120	0.018	0.010
								0.180	0.701	**0.000**
								0.331	0.163	0.297
								0.028	0.534	0.024
								0.009	**0.000**	**0.000**
								0.369	0.457	0.005
LJ									0.058	**0.000**
									0.025	**0.000**
									0.921	0.846
									0.062	**0.000**
									0.059	0.024
									0.084	0.249
EB										0.048
										0.000
										0.809
										0.005
										0.467
										0.003

Tests to compare the allelic structure of the eleven subpopulations follow the same trend as those for the genotypic structure and show even more distinctive differentiation patterns (giving 37 additionally significant comparisons at a 5 % level, mainly at the PGI and PGM loci, detailed results are listed in Table 34).

Table 34. Tests of allelic differentiation between pairs of eleven subpopulations of *P. obliterata* sampled in four biotope types. Results per locus (PGI, PGM, ME, GOT, PK, ACP) were listed according to Table 35 for each location. Bold numbers consider significant differences between populations accepting a 5 % significance level (applying sequential Bonferroni procedure).

	Subpopulation									
	Habitat type									
	Várzea			**Igapó**				**Mixed-water**	**Terra firme**	
	IP	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
	0.034	**0.000**	0.217	**0.000**	**0.000**	**0.000**	0.416	0.002	0.016	**0.000**
	0.003	**0.000**	0.528	1.000	0.061	**0.000**	0.003	**0.000**	0.001	**0.000**
	0.000	0.572	1.000	0.026	0.748	0.031	0.286	0.164	0.585	0.002
	0.008	**0.000**	0.010	**0.000**	0.393	**0.000**	1.000	0.009	**0.000**	**0.000**
	0.046	0.664	0.062	0.578	0.206	0.063	0.222	0.524	0.039	0.004
IP		0.003	0.003	0.019	0.099	0.001	0.078	**0.000**	0.001	0.031
		0.324	0.210	0.036	**0.000**	0.005	0.307	0.244	0.006	**0.000**
		0.010	0.014	0.015	0.196	0.075	1.000	0.119	0.149	0.077
		0.000	**0.000**	**0.000**	**0.000**	**0.000**	0.001	**0.000**	**0.000**	**0.000**
		0.024	1.000	0.046	0.092	0.009	0.006	1.000	0.076	0.010
		0.199	1.000	0.262	0.301	0.222	0.149	0.277	0.315	0.037
MA			**0.000**	0.193	**0.000**	0.013	0.026	0.002	0.017	0.221
			0.003	0.006	**0.000**	**0.000**	0.003	0.015	**0.000**	**0.000**
			0.000	**0.000**	**0.000**	0.751	0.022	0.379	0.443	0.760
			0.789	0.001	0.885	0.100	0.477	0.014	1.000	0.007
			0.024	0.848	**0.000**	0.840	**0.000**	0.021	0.706	0.843
			0.248	1.000	0.612	0.463	0.672	0.506	0.254	0.002
IC				0.384	**0.000**	0.014	0.121	0.012	0.493	**0.000**
				0.054	**0.000**	**0.000**	0.477	0.017	0.290	**0.000**
				0.530	0.373	**0.000**	0.010	**0.000**	0.002	**0.000**
				0.007	0.885	0.045	0.375	0.068	0.791	0.004
				0.067	0.084	0.008	0.007	1.000	0.076	0.011
				0.298	0.368	0.333	0.182	0.366	0.414	0.037
PG					**0.000**	0.129	0.470	0.018	0.909	0.004
					0.000	0.002	0.010	0.036	0.024	**0.000**
					0.116	0.001	0.015	0.001	0.007	**0.000**
					0.001	**0.000**	**0.000**	0.285	0.003	**0.000**
					0.000	0.562	**0.000**	0.062	1.000	0.575
					0.898	0.485	0.902	0.482	0.373	0.004

	Subpopulation									
	Habitat type									
	Várzea			Igapó				Mixed-water	Terra firme	
	IP	MA	IC	PG	AV	TM	PQ	LJ	EB	AM
AV						**0.000**	0.026	**0.000**	**0.000**	**0.000**
						0.000	**0.000**	**0.000**	**0.000**	**0.000**
						0.003	0.183	0.013	0.016	0.002
						0.094	0.882	0.021	0.891	0.006
						0.000	0.388	0.126	**0.000**	**0.000**
						1.000	0.806	0.250	0.827	0.002
TM							0.001	0.017	0.021	0.001
							0.002	0.407	**0.000**	**0.000**
							0.158	0.876	0.967	1.000
							0.273	**0.000**	0.095	0.496
							0.000	0.011	0.449	1.000
							0.654	0.198	0.841	0.001
PQ								0.015	0.284	0.002
								0.043	0.073	**0.000**
								0.169	0.201	0.132
								0.004	0.560	0.029
								0.006	**0.000**	**0.000**
								0.198	0.518	**0.000**
LJ									0.001	**0.000**
									0.002	**0.000**
									1.000	0.721
									0.027	**0.000**
									0.067	0.013
									0.111	0.052
EB										0.001
										0.000
										0.804
										0.006
										0.456
										0.000

4.2.6.3 Genotypic Distinction of Biotope Types

Tests of genotypic discrimination between the four biotope types (Várzea, Igapó, mixedwater inundation forest and Terra firme; Table 35) revealed the strongest evidence for genetic differentiation between the Terra firme population and the inundation forest populations. In all pairwise comparisons (Terra firme versus Várzea, Igapó and mixedwater inundation forest) the genotypic structure of the enzyme locus PGM differed significantly ($P < 5$ % significance level corrected by the sequential Bonferroni procedure) between populations. Furthermore, at least one locus showed

additionally significant genetic differences (Terra firme/ Várzea: PK; Terra firme/ Igapó: PK and ACP; Terra firme/mixedwater: PGI and GOT).

Table 35. Tests of genotypic differentiation between pairs of populations of *P. obliterata* in the four biotope types. Bold numbers consider significant differences between populations at a 5 % significance level (applying sequential Bonferroni procedure).

		Biotope type		
Biotope type	**Locus**	**Igapó**	**Mixedwater**	**Terra firme**
Várzea	PGI	0.081	0.008	0.696
	PGM	**0.000**	0.013	**0.000**
	ME	0.892	0.057	0.031
	GOT	0.030	**0.002**	0.012
	PK	0.862	1.000	**0.001**
	ACP	0.071	0.618	0.172
Igapó	PGI		0.008	0.114
	PGM		0.339	**0.000**
	ME		0.029	0.011
	GOT		0.009	0.106
	PK		1.000	**0.001**
	ACP		0.013	**0.000**
Mixedwater	PGI			**0.001**
	PGM			**0.000**
	ME			0.929
	GOT			**0.000**
	PK			0.004
	ACP			0.563

However, populations from the different inundation forests (Várzea, Igapó, mixedwater) showed fairly homogeneous genotypic distributions, since hardly any significant differences across the three pairwise comparisons were found ($P < 5$ % significance level corrected by the sequential Bonferroni procedure; except for Várzea/Igapó, PGM and Várzea/mixedwater, GOT).

Even though significant differences in genotypic frequencies between the Terra firme and inundation forest populations were mainly revealed at the PGM and PK loci, no biotype specific genotypic combinations of these loci could be detected in the surveyed subpopulations. In respect of the locus PGM, the observed genotypic distinction between inundation forests and Terra firme can be attributed to an excess of different homozygotes in the respective subpopulations (all inundation forests subpopulations except for AV: PGM 3-3; Terra firme subpopulation AM: PGM 2-2).

Analogous tests for allelic differentiation between populations revealed the same trend (Table 36). Here, the populations from both Várzea and Igapó displayed additional genetic differentiation to the mixedwater inundation forest population at the PGI locus. To conclude, the results indicate a more pronounced differentiation between Terra firme and inundation forests than among the populations from the different inundation forests.

Table 36. Tests of allelic differentiation between pairs of populations of *P. obliterata* in the four biotope types. Bold numbers consider significant differences between populations at a 5 % significance level (applying sequential Bonferroni procedure).

		Biotope type		
Biotope type	**Locus**	**Igapó**	**Mixedwater**	**Terra firme**
Várzea	PGI	0.030	**0.000**	0.659
	PGM	**0.000**	0.005	**0.000**
	ME	0.884	0.044	0.014
	GOT	0.008	**0.000**	0.005
	PK	0.846	1.000	**0.000**
	ACP	0.100	0.564	0.134
Igapó	PGI		**0.001**	0.049
	PGM		0.361	**0.000**
	ME		0.033	0.010
	GOT		0.005	0.101
	PK		1.000	**0.000**
	ACP		0.021	**0.000**
Mixedwater	PGI			**0.000**
	PGM			**0.000**
	ME			0.902
	GOT			**0.000**
	PK			0.010
	ACP			0.558

4.2.7 Population Genetic Structure

To estimate the effect of geographic isolation of the subpopulation AM, the hierarchical AMOVA was analyzed twice, (1) including all eleven subpopulations (Table 37) and (2) for the ten subpopulations located in the vicinity of Manaus (Table 38).

Except for the 'among subpopulations/biotope' variance component in Table 38, neither ME nor ACP, though showing the same trend as the remaining loci, did provide any significant data. Therefore, only the results concerning the remaining four loci will be referred to in the following.

Table 37. Population genetic structure (effects of biotope type, ecological condition and geographic position) for eleven subpopulations of *P. obliterata* evaluated by a hierarchical analysis of molecular variance (AMOVA) based on the allele frequencies of six unlinked loci. Abbreviations: *df*, degree of freedom; *SS*, sum of squares; *V*, variation explained. [1]Biotope types: Várzea, Igapó, mixedwater inundation forest, Terra firme. [2]Ecological groups: inundation forest, upland plantation. [3]Regions: near Manaus (Amazonia State), at Amapá State. Significant (* $P < 0.05$; ** $P < 0.000$) effects are indicated in bold type.

Loci	PGI			PGM			ME			GOT			PK			ACP		
Variance component	*df*	*SS*	*V* (%)	*df*	*SS*	*V* (%)	*df*	*SS*	*V* (%)	*df*	*SS*	*V* (%)	*df*	*SS*	*V* (%)	*df*	*SS*	*V* (%)
Among biotopes[1]	3	1.60	-1.22	3	5.65	1.62	3	0.29	-1.07	3	1.28	-0.01	3	2.01	-2.90	3	0.54	0.02
Among subpopulations/biotope	7	6.45	**4.03****	7	9.21	**9.16****	7	1.38	2.02	7	2.89	**7.09***	7	8.52	**13.34****	7	1.23	-0.18
Within subpopulations	385	132.36	**97.19****	385	110.26	**89.22****	319	38.92	99.05	305	39.81	**92.91***	316	70.89	**89.55****	334	62.27	100.16
Among ecological groups[2]	1	0.36	-1.19	1	3.79	**5.78***	1	0.17	0.02	1	0.55	0.95	1	1.99	3.87	1	0.39	1.19
Among subpopulations/group	9	7.69	**3.51****	9	11.07	**8.03****	9	1.49	1.19	9	3.61	**6.72***	9	8.54	**9.46****	9	1.38	-0.58
Within subpopulations	324	132.36	**97.67****	385	110.26	**86.19****	319	38.92	98.79	305	39.81	**92.34****	316	70.89	**86.67****	334	62.27	99.38
Among regions[3]	1	0.88	0.27	1	6.41	24.20	1	0.13	-0.60	1	0.74	4.37	1	1.12	0.67	1	1.08	9.32
Among subpopulations/region	9	7.16	**3.03***	9	8.45	**4.47****	9	1.53	1.32	9	3.42	**6.01***	9	9.41	**10.87****	9	0.68	-1.73
Within subpopulations	324	132.36	**96.71****	385	110.26	**71.33****	319	38.92	99.28	305	39.81	**89.62***	316	70.89	**88.46****	334	62.27	92.42
Among all subpopulations	10	8.04	**3.08****	10	14.86	**10.46****	10	1.66	1.20	10	4.16	**7.09****	10	10.53	**11.05****	10	1.77	-0.17
Within subpopulations	324	132.36	**96.92****	385	110.26	**89.54****	319	38.92	98.80	305	39.81	**92.91****	316	70.89	**88.95****	334	62.27	100.17

Table 38. Population genetic structure (effects of biotope type and ecological condition) for ten subpopulations of *P. obliterata* in the vicinity of Manaus, Amazonia, Brazil evaluated by a hierarchical analysis of molecular variance (AMOVA) based on the allele frequencies of six unlinked loci. Abbreviations: *df*, degree of freedom; *SS*, sum of squares; *V*, variation explained. [1]Biotope types: Várzea, Igapó, mixedwater inundation forest, Terra firme. [2]Ecological groups: inundation forest, upland plantation. Significant (* $P < 0.05$; ** $P < 0.000$) effects are indicated in bold type.

Loci	PGI			PGM			ME			GOT			PK			ACP		
Variance component	*df*	*SS*	*V* (%)	*df*	*SS*	*V* (%)	*df*	*SS*	*V* (%)	*df*	*SS*	*V* (%)	*df*	*SS*	*V* (%)	*df*	*SS*	*V* (%)
Among biotopes[1]	3	1.59	-1.48	3	2.36	-1.31	3	0.18	-2.16	3	0.79	-2.02	3	0.93	-7.00	3	0.16	-0.20
Among subpopulations/biotope	6	5.58	**4.17****	6	6.09	**6.63***	6	1.36	**3.10***	6	2.63	**6.95***	6	8.48	**16.37****	6	0.53	-1.68
Within subpopulations	293	119.07	**97.31****	355	105.42	**94.69****	289	33.82	99.06	276	39.81	**95.07***	288	64.33	**90.63****	306	53.86	101.88
Among ecological groups[2]	1	0.35	-2.22	1	0.51	-2.78	1	0.06	-1.92	1	0.07	-4.66	1	0.91	-1.69	1	0.01	-0.45
Among subpopulations/group	8	6.81	**3.59****	8	7.95	**6.44****	8	1.47	1.93	8	3.36	**6.62***	8	8.50	**11.51****	8	0.67	-1.73
Within subpopulations	293	119.07	**98.63****	355	105.42	**96.34****	289	33.82	99.98	276	39.81	**98.04***	288	64.33	**90.18****	306	53.86	102.19
Among all subpopulations	9	7.16	**3.07****	9	8.45	**5.62****	9	1.53	1.50	9	3.42	**5.42***	9	9.41	**11.00****	9	0.69	-1.83
Within subpopulations	293	119.07	**96.93****	355	105.42	**94.38****	289	33.82	98.50	276	39.81	**94.58***	288	64.33	**89.00****	306	53.86	101.83

The proportion of the 'among regions' variance component in Table 37 is not significant. In other words, the allelic variation is not explained by separating the subpopulations into regional groups (Manaus region versus Amapá State).

Assorting the subpopulations according to their biotope type (Várzea, Igapó, mixedwater inundation forest or Terra firme) did not account for any significant fraction of the allelic variability either in all eleven or the ten local subpopulations at Manaus.

Since the three inundation forests biotopes show very similar environmental conditions (Chapter 3.1.1), the data were reanalysed with the subpopulations classified according to their ecotype (inundation forest versus upland plantation). Including AM (Table 37), most of the allelic diversity is still found within subpopulations, but a small proportion separates the ecotypes at the PGM locus. However, if AM was excluded from the analysis (Table 38), leaving only EB as an upland plantation subpopulation, this significant portion at the PGM locus was absent.

The observed allelic diversity within subpopulations (approx. 90 % or more) was generally much higher than the differentiation between subpopulations (appreciable values of approx. 10 % only at the PGM locus, Table 37, and the PK locus, Tables 37 & 38).

4.2.8 Isolation by Distance and Effective Number of Migrants

Interpretation of ecological differentiation requires information concerning the geographic distance between the subpopulations (Rousset 1999). The linear geographic distances between pairs of eleven subpopulations are presented in Table 39. AM was located approximately 1,000 km east of the geographically closer subpopulations in the vicinity of Manaus (Fig. 50), which were separated by 46.6 km on average, with the linear distance ranging from 7.4 to 104.7 km (Table 39).

Given that AM is most likely highly isolated from the remaining subpopulations, it was excluded from the examined migration model. Thus only ten subpopulations around Manaus were considered for the test of isolation by distance. No significant correlation (P > 5 % significance level) between the geographic distance of those subpopulations and their genetic distance as evaluated by the estimated level of gene flow M $[(1-F_{ST})/(2F_{ST})]$ was evident (Fig. 52).

Table 39. Linear geographic distances [km] between pairs of eleven sample sites in four biotope types. Abbreviations: MW, mixed water; TF, Terra firme.

	Subpopulation									
	Habitat type									
	Várzea			**Igapó**				**MW**	**TF**	
	IP	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	7.4	25.8	66.3	55.3	54.3	38.4	45.5	22.8	55.5	1079.3
IP		27.4	68.4	48.2	45.0	32.4	45.0	22.4	51.6	1080.8
MA			40.9	68.6	62.5	35.4	21.4	8.1	39.7	1035.7
IC				104.7	97.0	65.5	29.7	46.9	51.6	1013.2
PG					9.3	40.3	75.4	60.7	63.2	1104.5
AV						32.0	67.5	54.5	54.1	1095.2
TM							35.8	27.8	25.1	1066.2
PQ								22.8	25.4	1036.5
LJ									35.7	1058.4
EB										1041.7

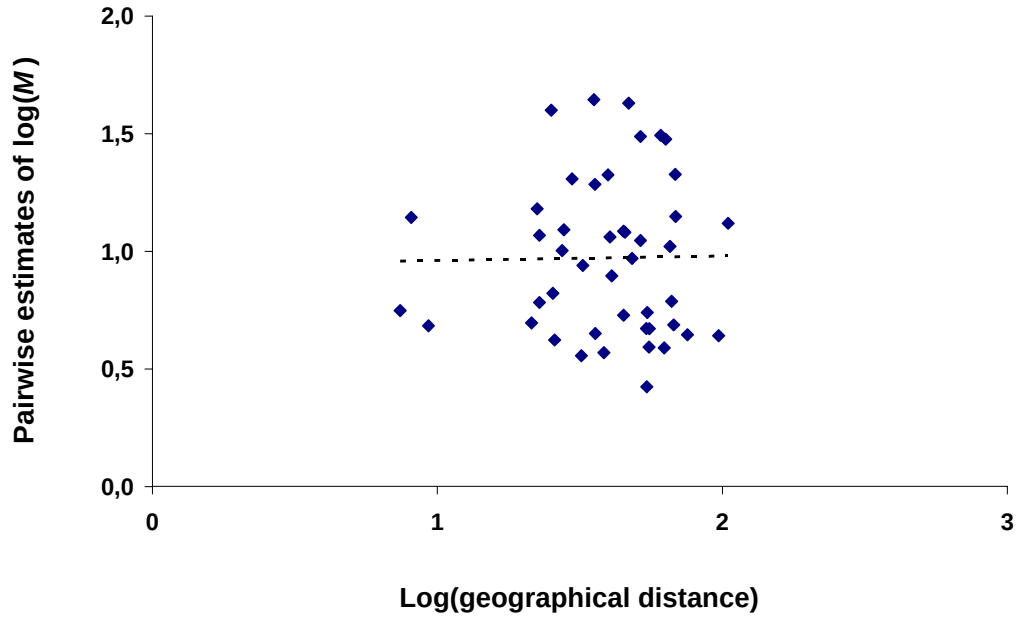

Figure 52. Relation of genetic and geographic distance for ten subpopulations of *P. obliterata* in the vicinity of Manaus, Brazil. Pairwise estimated levels of gene flow M $[(1-F_{ST})/(2F_{ST})]$ are shown as a function of the linear geographic distance d [km] separating two subpopulations.
$\text{Log}_{10}(M)$ is plotted against $\log_{10}(d)$. The line was generated using a conventional linear regression: $\log_{10}(M) = 0.94 + 0.02 \log_{10}(d)$ with an R^2 value of 0.0002.

Since *M* can be used to test particular hypotheses about pathways of dispersal (Slatkin 1993), the mean values of *M* within and between the different biotope types were estimated (Table 40). Standard deviations were always very high, indicating a heterogeneous pattern of gene flow between the respective subpopulations. Within the various biotopes, *M* was most pronounced in the Várzea. The amount of gene flow between biotopes was even more distinct, with the highest values being revealed among the mixedwater inundation forest and both Várzea and Igapó. Estimates of *M* between inundation forests and Terra firme were almost equal to those among Várzea and Igapó. However, the extent of gene flow among the inundation forests and the geographically remote plantation site AM was much lower than the estimated level between the inundation forests and the closer plantation EB.

Table 40. Mean value and standard deviation of the estimated level of gene flow *M* within and between different biotope types (estimated $M = (1-F_{ST})/(2F_{ST})$; Slatkin 1993). Abbreviations: MW, mixed water; TF, Terra firme; EB, subpopulation Embrapa; AM, subpopulation Amapá.

Within biotope types					
Várzea	9.2 ± 6.2				
Igapó	5.6 ± 2.9				
MW	-				
TF	4.9				
Between biotopes types					
	Igapó	MW	TF (EB, AM)	TF (EB)	TF (AM)
Várzea	10.8 ± 10.2	19.4 ± 16.0	9.8 ± 10.7	16.9 ± 11.4	2.6 ± 0.8
Igapó		15.2 ± 11.1	11.8 ± 14.5	20.3 ± 17.4	3.4 ± 1.1
MW			11.0 ± 11.6	19.0	2.8

The effective number of migrants (*Nm*) for the ten subpopulations in the vicinity of Manaus was 2.03 (estimated by the software GENEPOP). Considering only the nine inundation forest locations, the average number of migrants exchanged between local subpopulations was 2.07. Since *Nm* should be higher for the remaining locations, when an isolated location is removed, these results suggest a hardly isolated position of the Terra firme subpopulation EB. Both values of *Nm* indicate high levels of gene flow between the studied localities and are sufficient to prevent genetic differentiation through the effect of genetic drift alone (Slatkin 1985; Wright 1931).

4.2.9 Cluster Analysis of Genetic Similarity

The mean value for Nei's genetic distance *D* (Nei 1978) between the eleven subpopulations of *P. obliterata* was 0.070 ± 0.048 (Table 41). Within the different biotopes, the values ranged from 0.058 ± 0.024 in the Várzea and 0.077 on the Terra firme to 0.080 ± 0.022 in the Igapó. The mixedwater inundation forest subpopulation LJ was genetically close to the remaining inundation forest subpopulations (0.039 ± 0.022). The geographically remote Terra firme subpopulation AM was also the genetically the most distinct one from the inundation forest subpopulations (0.145 ± 0.046), whereas the closer Terra firme subpopulation EB was genetically similar (0.038 ± 0.028). However, some inundation forest subpopulations, e.g. AV (0.092 ± 0.021) and JC (0.080 ± 0.032), were genetically remote from the others as well.

Table 41. Nei's (1978) genetic distance reconstructing measure between pairs of eleven subpopulations of *P. obliterata* sampled in four biotope types. Abbreviations: MW, mixed water; TF, Terra firme.

	Subpopulation									
	Biotope type									
	Várzea			**Igapó**				**MW**	**TF**	
	IP	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	0.069	0.095	0.056	0.100	0.126	0.103	0.026	0.064	0.079	0.225
IP		0.050	0.022	0.058	0.075	0.054	0.027	0.036	0.042	0.152
MA			0.056	0.038	0.107	0.008	0.080	0.040	0.018	0.112
IC				0.035	0.086	0.040	0.016	0.009	0.014	0.151
PG					0.089	0.046	0.084	0.018	0.018	0.132
AV						0.111	0.067	0.075	0.083	0.088
TM							0.083	0.041	0.010	0.091
PQ								0.027	0.053	0.196
LJ									0.025	0.162
EB										0.077

Since Nei's (1987) *D* depends on allele frequencies, the genetic distances were additionally estimated based on genotype frequencies (Tomiuk & Loeschcke 1991). The genotypic distances between the eleven subpopulations were lower but revealed the same trend as the genetic distances according to Nei (1987), except for JC being the most distant subpopulation to all remaining ones (Table 42).

Table 42. Tomiuk & Loeschcke's (1991) genetic distance reconstructing measure between pairs of eleven subpopulations of *P. obliterata* sampled in four biotope types. Abbreviations: MW, mixed water; TF, Terra firme.

	Subpopulation									
	Biotope type									
	Várzea			**Igapó**				**MW**	**TF**	
	IP	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	0.068	0.070	0.063	0.063	0.078	0.085	0.073	0.080	0.062	0.050
IP		0.027	0.036	0.038	0.008	0.031	0.007	0.028	0.032	0.049
MA			0.028	0.020	0.015	0.017	0.013	0.008	0.005	0.051
IC				0.009	0.013	0.054	0.019	0.036	0.015	0.046
PG					0.011	0.032	0.023	0.028	0.014	0.065
AV						0.015	0.014	0.024	0.015	0.064
TM							0.036	0.011	0.032	0.056
PQ								0.024	0.011	0.033
LJ									0.022	0.050
EB										0.033

To illustrate the genetic relationship between the subpopulations, cluster analyses were computed by the neighbour-joining method, which is generally superior in obtaining a correct phylogenetic tree topology compared to other reconstructing methods (Saitou & Nei 1987; Jin & Nei 1991). The resulting dendrograms based on Nei's (1987) and Tomiuk & Loeschke`s (1991) genetic distance, respectively, are presented in Figures 53 & 54. Both dendrograms did not reveal any distinct grouping pattern related to the geographic position or associated river type of the inundation forest subpopulations, except for LJ being situated between MA and TM (Fig. 54; compare geographic positions in Fig. 50). However, the Terra firme subpopulations (EB, AM) were positioned somewhat discrete from most of the inundation forest subpopulations (Fig. 54).

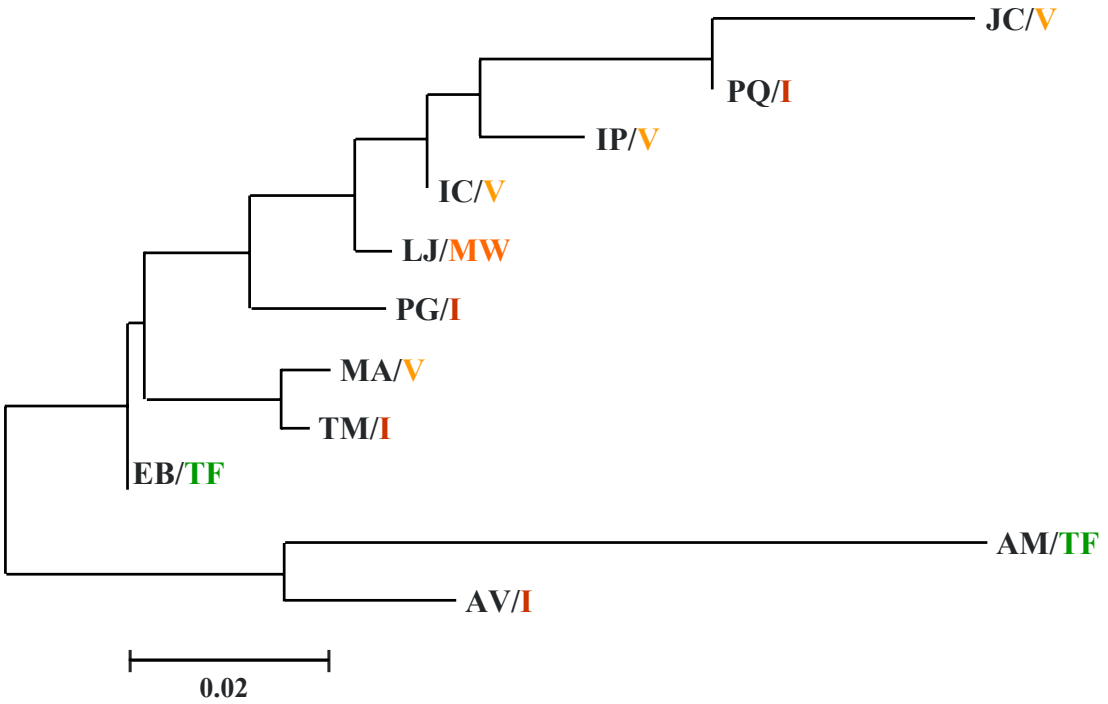

Figure 53. Dendrogram (neighbour-joining) of eleven subpopulations of *P. obliterata* based on Nei's (1987) genetic distance (Table 41). The sample sites are listed in Table 17. Biotope types: V, Várzea; I, Igapó; MW, mixedwater inundation forest; TF, Terra firme.

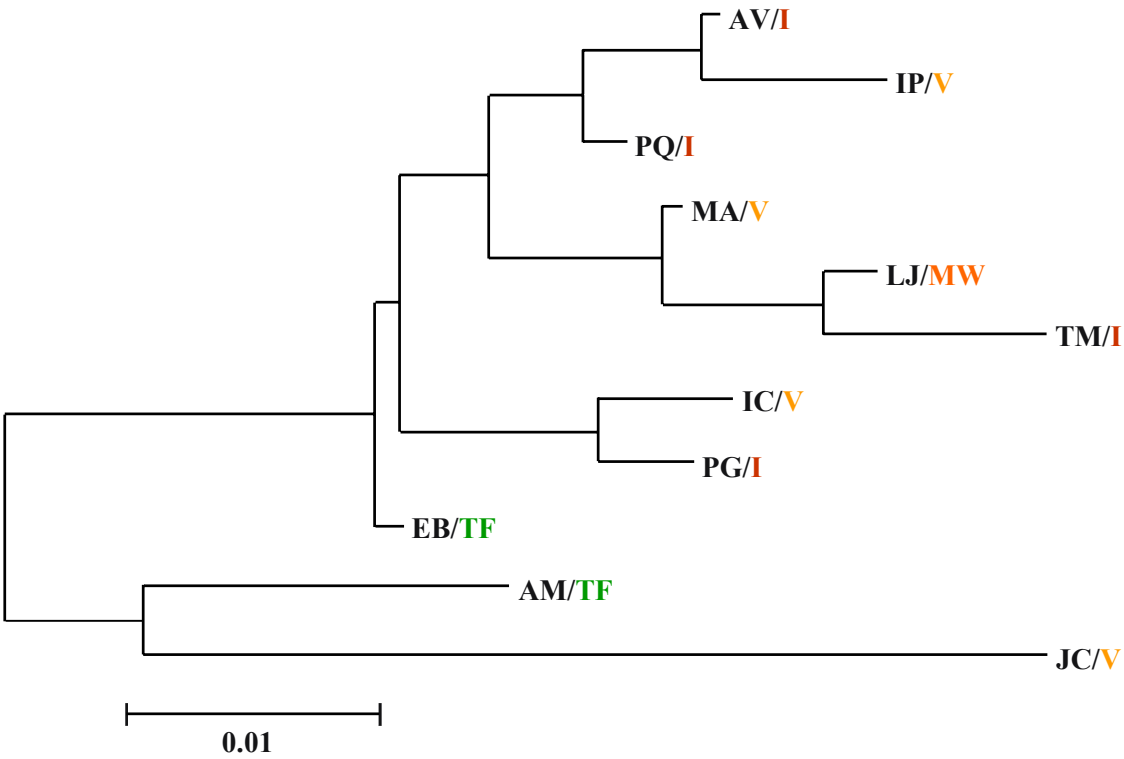

Figure 54. Dendrogram (neighbour-joining) of eleven subpopulations of *P. obliterata* based on Tomiuk & Loeschke's (1991) genetic distance (Table 42). The sample sites are listed in Table 17. Biotope types: V, Várzea; I, Igapó; MW, mixed water inundation forest; TF, Terra firme.

4.3 Discussion

4.3.1 Population Dynamics and Adaptation in *P. obliterata*

4.3.1.1 Concept of Dispersal

The millipede *P. obliterata* is thought to have evolved in the Peruvian Andes (Golovatch & Sierwald 2001) and to have subsequently invaded inundation forests, where, like some other native terrestrial invertebrates (Adis 1997), it became adapted to regularly flooded biotopes. First taken in 1956 from an island in the Solimões River near Iquitos, Peru (Kraus 1960), at present *P. obliterata* is evidently a species widespread all over Amazonia, i.e. Peru, Colombia and Brazil (Golovatch & Sierwald 2001).

Although specimens move actively over short distances, e.g. when climbing tree trunks (Chapter 3.2.3.1.1), the natural dispersal capacity of small millipedes is fairly low (Hopkin & Read 1992). Flowing waters can transport invertebrates passively over considerably greater distances than can be traversed using their own locomotory apparatus (Wang & Schreiber 1999). Passive dispersal of invertebrates on floating objects has frequently been reported (Hoskin 1999; Aliani & Molcard 2003; Gutow 2003), also unidirectional drift of terrestrial organisms in riverine ecosystems (Dörge et al. 1999; Tenzer & Plachter 2003). In the Amazonian floodplains, large rivers like Solimões, Amazon and Negro apparently serve as pathways of repeated downstream dispersal for individuals of *P. obliterata* on floating tree trunks and branches, as also suggested for the congener *P. insularis* (Bergholz et al. 2005). Support for this can be found in the study of another Central Amazonian millipede, *Pycnotropis tida*, where the authors postulated a similar scenario of downstream colonisation along the Solimões and Amazon River (Bachmann et al. 1998; Golovatch et al. 1998). Accordingly, the subpopulations of *P. obliterata* within the Várzea and Igapó are expected to be connected by gene flow and thus to be genetically rather similar. Since the precise origin of the *P. obliterata* subpopulations along the Negro River is yet unknown, the relatedness of populations from Igapó and Várzea still needs to be clarified. Given their spatial isolation, the respective subpopulations from Várzea and Igapó upstream of Manaus, i.e. at the confluence of both river systems (Fig. 50), are assumed to be genetically distinct. In contrast, subpopulations within the mixedwater area and further downstream the Amazon River could either represent a mixture of individuals from both Várzea and Igapó or be dominated by specimens of a single inundation forest type.

Besides being dispersed by rivers, the species *P. obliterata* has also been transported widely by human traffic, even to European hothouses (Adis et al. 2001; Golovatch & Sierwald 2001), and is hence living in a variety of biotopes that range from natural woodlands to man-made plantations. The studied plantations, EB and AM (Fig. 50), are located 35 km and 60 km away from the main rivers Negro and Amazon, respectively. Therefore, the established populations could have been introduced by man with agricultural plant material, e.g. palm seeds, as suggested for the congener *Myrmecodesmus hastatus* (Bergholz et al. 2004).

In the cultivated upland area of the CPPA/Embrapa near Manaus, specimens of *P. obliterata* were first collected in 1997 on a palm tree plantation of *Bactris gasipaes* Kunth (Araceae) (Adis et al. 2001). The seeds of the cultivated palms and therewith probably numerous concealed opportunists, millipedes included, were imported approximately 20 years ago from Peru (Garcia, pers. commun.). Apparently, some individuals subsequently established a subpopulation on the adjacent banana plantation EB (*Musa* spec.; Musaceae). Since the saplings of the respective plantation originate from the eastern coast of Brazil (Bahia State; Garcia, pers. commun), it is rather unlikely that they hosted any specimens of *P. obliterata*. The subpopulation samples from Amapá State were collected in decomposing seed piles on the oil palm plantation AM (*Elaeis guineensis* Jacq.; Araceae). Again, *P. obliterata* were almost certainly brought along with palm seeds imported from Peru to establish the respective plantation in 1960 (Oliveira, pers. commun.). Whereas *P. obliterata* on the secluded site AM are apparently isolated and thus may be genetically distinct from Central Amazonian populations, the specimens on the local site EB might be connected to inundation forest populations via gene flow. Since the plantation is situated only circa 15 km away from the closest tributary of the Negro River (Fig. 50), some individuals native to the Igapó may sporadically have been brought along with transported plant material (e.g. seeds, logs).

4.3.1.2 Adaptation Hypothesis

The millipede *P. obliterata* is believed to have evolved in the Andean uplands and so faces ideal conditions on Amazonian upland plantations. In contrast, individuals living in the inundation forests were forced to adapt to their regularly flooded biotopes, apparently representing a different ecotype based on behavioural traits (Chapter 3.3).

A relation between genotypes and the occupancy of upland versus floodplain forests has already been described for the millipede, *P. tida* (Bachmann et al. 1998; Golovatch et al. 1998), and an insect, *Neomachilellus scandens* (Wolf & Adis 1992), in the study area. Therefore, the respective populations of *P. obliterata* are also assumed to be differentiated genetically, possibly representing different ecological races, subspecies or, as in *N. scandens* (Wolf & Adis 1992), even species.

4.3.1.3 Population Genetic Implications from Allozyme Data

Do the obtained genetic data support the colonisation hypotheses, i.e. repeated downstream migration along the rivers and introductions by man on plantations, as well as the presumed adaptation model, i.e. ecological differentiation of *P. obliterata* populations in seasonally flooded biotopes?

4.3.1.3.1 Random Mating and Outcrossing

All surveyed subpopulations of *P. obliterata* exhibited heterozygote deficiencies but few also an excess of heterozygotes at individual loci (Table 24). Five subpopulations (IP, IC, PG, AV and EB) displayed significant deviations from Hardy-Weinberg expectations at respective single loci (Table 25), this being caused by a significant lack of heterzygotes. Three (IP, IC and AV) of these subpopulations were located on islands, whereas the others were situated on the shore of the Negro River (PG) or an upland plantation (EB).

Genotype proportions in the Hardy-Weinberg equilibrium can only be expected if mating is random and forces such as selection, migration and mutation are absent (Weir 1996). Accordingly, deficiency of heterozygotes may be explained by natural selection against hybrids, assortative mating, inbreeding, or by population mixing (Wahlund 1928; Pavlíček et al. 1997).

Biotope specific selection against heterozygous hybrids of different ecotypic forms (outbreeding depression) can result in a notable excess of homozygotes. With such selection acting however, one would expect the inundation forest subpopulations close to upland areas (TM, PQ, JC, PG; Fig. 50) to be the most affected, which has only been observed in a single case (PG). Since the animals from different populations are morphologically identical (Chapter 3.2.2.1), mate choice based on resembling phenotypes (Hartl & Clark 1997) cannot explain the phenomenon satisfactorily either.

In this context, assortative mating may rather be related to ecologically important traits (Dieckmann & Doebeli 1999), such as breeding behaviour (Seehausen et al. 1997). Yet individuals within inundation forests seem to reproduce only during the terrestrial phase, just as those on upland plantations (Chapter 3.3.2.3). The excess of homozygotes might also be caused by inbreeding due to small local breeding units, as previously described for another millipede, *Tetrarthrosoma syriacum* (F_{IS} = 0.422 to 0.541) (Pavlíček & Nevo 1996). In *P. obliterata*, the mean inbreeding coefficient, F_{IS} = 0.131 ± 0.067 (Table 29), reveals a significant lack of heterozygotes within subpopulations, particularly in the inundation forests. Here, one could assume that small demes (i.e. semi-isolated subpopulations, Hartl & Clark 1998) were originally established from few colonisers, thus accounting for substantial mating to close relatives. However, the postulated demes would be characterised by a loss of genetic variability as a result of genetic drift (Sperlich 1988), with parameters such as allelic richness being susceptible to a decrease (Nei et al. 1975; El Mousadik & Petit 1996). The gene pool of such populations may easily be distinguished by the occurrence of frequent private alleles (Slatkin 1985). In contrast, the genetic diversity in *P. obliterata* is high (Chapter 4.3.2), with hardly any private alleles (IP: ACP 4; PG: PGM 6; Table 24) found. This implies a large effective population size due to high levels of gene flow, preventing inbreeding within subpopulations (Chen et al. 2004). A large proportion of the genetic variation (50 to 90 % according to average pairwise differences and AMOVA respectively; Tables 30a-f & 37) was found within subpopulations rather than among subpopulations. This result is compatible with the interpretation that outcrossing rates in natural subpopulations of *P. obliterata* may be relatively high (Jorgensen & Mauricio 2004). Along with multiple introductions, repeated genetic exchange could help in accounting for the maintenance of considerable levels of diversity (Petit et al. 2004).

These considerations suggest that the excess of homozygotes is due to population mixing (Wahlund effect; Wahlund 1928). Under the assumption of mixing of formerly isolated subpopulations, the analysed samples per location could actually be composed of individuals originating from demes with different genotypic composition. According to field observations, the millipedes apparently cease to reproduce during the aquatic phase, when specimens were sampled (Chapter 3.3.2.3). The respective mixture of adults was most likely taken before hybridisation had occurred in the pooled

subpopulation and thus comprised an excess of homozygotes compared to panmictic proportions (Hartl & Clark 1989; Chevillon et al. 1998).

The fact that there is no consistent deficit of heterozygotes across loci and locations in the subpopulations of *P. obliterata* points to an important distinction between subdivision and inbreeding. Even though no significant genotypic linkage exists between loci (Table 28), a reduction in heterozygote frequencies resulting from inbreeding should be the same for all loci (Hartl & Clark 1989; Goudet et al. 1996; Pavlíček et al. 1997; Valles-Jimenez et al. 2005). With population subdivision, a particular heterozygous genotype being present in excess or deficit depends on the covariance of allele frequencies across populations (Hartl & Clark 1989). Concordantly, an excess of heterozygotes at individual loci was revealed within some samples of *P. obliterata* (Table 24). The subpopulations showing heterozygote deficiency thus appear to be a mixture of resident and immigrated specimens. That is consistent with the presumed passive dispersal events in the inundation forests, whose subpopulations seem to display the plainest deficiency of heterozygotes (Table 29).

4.3.1.3.2 Gene Flow and Population Subdivision

Wright's standardised variance F_{ST} was used to calculate gene flow between Central Amazonian subpopulations of *P. obliterata*. The effective number of migrants, *Nm*, has been shown to give reasonably accurate estimates under a variety of conditions (Slatkin 1985). Values of more than one immigrant into the average deme per generation suggest sufficient gene flow to prevent differentiation due to random drift (Wright 1931), regardless of population size (Ellstrand & Elam 1993). The number of migrants exchanged among inundation forest subpopulations of *P. obliterata*, $Nm = 2.07$ (Chapter 4.2.8), demonstrates a substantial level of gene flow (Slatkin 1985) over occasionally long distances (7.4 to 104.7 km linear distance; Table 39), sufficient to prevent isolation by distance (Fig. 52). In fact, any correlation between the genetic and geographic distance was only observed on larger scales, when the remote subpopulation from Amapá State was taken into consideration (located approx. 1,000 km to the east, Fig. 50; genetically most distinctive, Tables 31 & 41). These results additionally support the hypothesis of multiple introductions of *P. obliterata* during colonisation along the two rivers in Central Amazonian inundation forests.

Since no isolation events but relatively large values of gene flow *M* (Table 40) were found, *P. obliterata* might have only recently colonised the area, and the time spent in the present range could have been insufficient to approach an equilibrium between genetic drift (as a diversifying factor) and gene flow (as a homogenising factor) (Slatkin 1993). Most of the natural groups of populations are probably not at equilibrium (McCauley 1993; Hutchison & Templeton 1999; Kinnison et al. 2002). *Nm* estimates, however, depend on the assumption that the species under study is at genetic and demographic equilibria. If subpopulations are continually going extinct and being recolonised, true demographic equilibrium does not exist and *Nm* estimates depend additionally on the extinction rate (Slatkin 1985). In the case of allozyme frequencies not being at equilibrium, it is impossible to distinguish whether the patterns reflect current gene flow or historical dispersal events (Avise 1994; Uthicke & Benzie 2000; Ketmaier 2002). Population history can produce patterns similar to the effects of ongoing gene flow (Felsenstein 1982; Templeton et al. 1995; Hewitt 2000). There is no basis for distinguishing between events of the migration model assumed and any other evolutionary scenario that could lead to the same pattern of gene frequencies within and among populations (Cockerham & Weir 1993). Besides, due to interactions of selection and drift with gene flow, the effective migration rate is highly variable across the genome. Heterosis, i.e. local heterogeneous selection, can increase the frequency of immigrant alleles, whereas 'background' selection against weakly deleterious alleles can effect the opposite (Charlesworth et al. 1993; Ingvarsson & Whitlock 2000). In genome regions with low recombination, a neutral locus linked to a locus under selection will likewise be affected, resulting in frequencies other than those predicted from neutral expectations (Ingvarsson & Whitlock 2000). In addition, rare dispersal events are thought to have a disproportionately large influence on the differentiation among populations (Slatkin 1985; Zhivotovsky et al. 1994; Ingvarsson & Whitlock 2000). Since multiple interpretations of population structure are possible, comparative methods such as *F*-statistic and Nei´s (1978) genetic distance *D* are adequate for determining the relative contribution of gene flow (Bohonak 1999).

As revealed by the mean fixation index, F_{ST} = 0.079 ± 0.052 (Table 31), only approximately 8 % of the genetic variation is ascribable to genetic differences between subpopulations. Accordingly, Nei´s (1978) mean genetic distance between subpopulations, *D* = 0.070 ± 0.048 (Table 41), indicates population subdivision but reveals no subspeciation (Sperlich 1988). The genetic differences between inundation

forest populations were even less pronounced (F_{ST} = 0.068 ± 0.039; D = 0.059 ± 0.031). The observed divergence in *P. obliterata* appears to be low for an animal species that lacks efficient means for active long-distance locomotion. This becomes even more apparent in comparison to other soil-dwelling invertebrates, e.g. certain snail species in Central Germany, whose populations are genetically distinct across distances of less than 1 km (Pfenninger et al. 1996). Other studies of different taxa also reported stronger genetic differentiation for species with limited dispersal activities (references in Bohanak 1999). Populations of other millipedes, e.g. *Nemasoma varicorne*, F_{ST} = 0.277 ± 0.12 (Jensen et al. 2002), and *P. tida*, F_{ST} = 0.315 (Bachmann et al. 1998; Golovatch et al. 1998), were highly differentiated over their examined range. *T. syriacum* even displayed microgeographic divergence (F_{ST} = 0.028; D = 0.013-0.131) at distances of less than 200 m (Pavlíček & Nevo 1996). Allopatric populations of *Stygiochiropus communis* showed major genetic differences (D = 0.400), mostly explained by the presence of isolated provinces (Humphreys & Adams 2001; Humphreys & Shear 1993). Data for *Glomeris* taxa, however, are contradictory. Substantial differentiation (D = 0.01-0.41) according to geographic distances could be observed within four different *Glomeris* species (Hoess et al. 1997; Hoess & Scholl 2001), e.g. *G. hexasticha*, D = 0.09 at a distance of 190 km (Hoess et al. 1997). Such a correlation was not evident within four other species which comprised only minor genetic variation over large parts of their respective geographic ranges (Hoess et al. 1997; Hoess & Scholl 1999).

The rather low magnitude of differentiation in *P. obliterata* is consistent with the estimated level of gene flow. The passive mode of translocation via waterways seems to effectively promote long-distance dispersal events within the inundation forests. Repeated introductions, particularly if they come from disparate portions of a large native range, ameliorate genetic diversity (Ingvarsson 2001; Lambrinos 2004). The same has been reported for another colonising species, the mosquito *Aedes albopictus* invasive to the United States (Kambhampati et al. 1990). Individuals of *P. obliterata* when cast ashore evidently serve as a genetic supply for the genetically rather homogeneous subpopulations on islands and along banks, sufficient to prevent genetic differentiation due to genetic drift (Sperlich 1988; Wright 1978). The heterozygous offspring of immigrants may have higher fitness than the resident individuals, resulting in a higher effective migration rate (Ingvarsson & Whitlock 2000).

4.3.1.3.3 Unidirectional Dispersal and Biotope-Related Differentiation

Although passive dispersal by river is unidirectional in nature, the interference with currents does not provide for a strictly homogeneous distribution. Hence individual subpopulations may receive fewer immigrants and thereby become relatively isolated. Consequently, they display distinctive genotypic frequencies, as is obvious in the fairly distinct subpopulations JC (F_{ST} = 0.096 ± 0.036, D = 0.080 ± 0.032) and AV (F_{ST} = 0.101 ± 0.025, D = 0.092 ± 0.021; Tables 31 & 41) that both comprise of values below the average estimated gene flow (JC: M = 5.1 ± 2.9; AV: M = 4.4 ± 0.9; Table 40).

According to the tests for genotypic discrimination between the four biotopes, i.e. white-, black-, and mixedwater inundation forests as well as upland areas (Table 35), the populations within different types of inundation forests display a strong genetic cohesion.

The genetic similarity of the Várzea and Igapó populations is likely to be based on historical events, since gene flow between the respective rivers systems is restricted: an exchange of migrants via waterway occurs mainly downstream of Manaus, where the rivers Negro and Solimões merge (Fig. 50). Therefore, the genetic homogeneity of the two populations is better explained by recent common decent. They appear to originate from the same ancestral upstream population at the Solimões River near Iquitos, Peru (cf. Kraus 1960). Sediment analyses at the northern banks of the Negro River in the vicinity of the Igapó subpopulation PG (upstream of Manaus, where the two rivers meet) indicate a former inflow of waters from the Solimões River (Furch 1999). Here, inflow of whitewater is believed to occur during high water periods via the numerous tributaries flowing through the lowlands separating the two rivers (Irion, pers. commun.; cf. Fig. 50). Such incidents may have introduced the individuals that established the present subpopulations at the lower Negro River (PG and AV), and further downstream (TM, PQ). Nevertheless, gene flow from the source populations in Peru is mostly confined to subpopulations along the Solimões/Amazon River, as reflected by both the less pronounced differentiation (according to F_{ST}, D; Table 31 & 41) and higher estimates of gene flow (M; Table 40) within the Várzea. In contrast, the subpopulations along the Negro River have apparently been exposed to recent genetic drift, since some rare alleles (PGI 3 and ACP 3; Table 24) are even scarcer in the Igapó.

The subpopulation LJ in the mixedwater inundation forest, i.e. the area, where the two rivers unite at present (Fig. 50), is evidently composed of individuals from both Várzea and Igapó (cf. cluster analysis, Fig. 54: medial position of LJ within the inundation forests). LJ is genetically most similar (F_{ST} = 0.042 ± 0.026, D = 0.039 ± 0.022; Tables 31 & 41) to the subpopulations of either biotope and comprises a high proportion of heterozygotes (H = 0.484; Table 24). The latter is characteristic of a mixed population in which hybridisation occurs continuously (Hartl & Clark 1989), probably due to constant gene flow from both Várzea and Igapó as shown by notably high values for M (Table 40).

In nature, both habitat quality and geographic distance can influence the differentiation between subpopulations to varying degrees (Pongratz et al. 2002). Habitat-related differentiation between two distinct groups of subpopulations with respect to within-group-differentiation decreases with distance (Rousset 1999). The relative effects of geography and ecology on genetic variation were hence evaluated by separate hierarchical analyses of molecular variance (AMOVA). The results show that allelic variation is neither explained by dividing the subpopulations into regional groups (Amazonia versus Amapá State; Table 37) nor according to their biotope type (Várzea, Igapó, mixedwater inundation forest or Terra firme; Tables 37 & 28). No allelic diversification among the subpopulations of *P. obliterata* with regard to geographic position or biotope type was detected. Yet the two factors (biotope and geographic isolation) are still not fully resolved because interactions between them cannot be unveiled (cf. Johannesson & Tararenkov 1997). For the PGM locus, a small proportion of allelic diversity separates subpopulations of floodplain (Várzea, Igapó, mixedwater) and upland (Terra firme) areas, but only if the remote upland subpopulation AM (approx. 1,000 km east, Fig. 50) was to be included in the analysis (Tables 37 & 38). These findings are consistent with the results of the tests for genotypic discrimination between the four biotopes, where significant differentiation between populations from upland plantations and inundation forests was also revealed at the PGM locus (Table 35). With only a single locus (PGM) being involved in the ecotype-dependent diversification, a contribution of ecological selection is possible. The effect of genetic drift would necessarily be more consistent among loci because it should affect all loci in the same way (Slatkin 1985). However, the genotypic differences between flooded and non-flooded biotopes are related to distinct allele frequencies (PGM 1, 4 and 5 are absent; Table 24) along with an excess of homozygotes (PGM 2-2) in the remote

subpopulation AM, whereas the upland subpopulation EB revealed no such deviation. AM also displayed different allelic and genotypic frequencies at other loci, e.g. at the GOT locus (GOT 2 and 3 are absent; Tables 24 & 33) and was genetically fairly distinctive (F_{ST} = 0.147 ± 0.047, D = 0.139 ± 0.048; Tables 31 & 41). Evidently, geographic isolation and subsequent genetic drift are responsible for the genetic constitution of AM. Support for this can be seen in the low levels of estimated gene flow (M; Table 40). In contrast, the regional subpopulation EB is genetically close to the inundation forest subpopulations (F_{ST} = 0.044 ± 0.034, D = 0.038 ± 0.028) and evidently connected via gene flow (Nm, Chapter 4.2.8; M, Table 40). The assumption that EB occasionally receives immigrants from adjacent inundation forests (Fig. 50) is consistent with the evidence that the nearby Igapó subpopulation TM is genetically the closest to EB (F_{ST} = 0.012, D = 0.010).

To summarise, the apparent ecotype-dependent diversification could just as well be the result of genetic drift in AM, but then the subpopulation AM is genetically more similar to EB (F_{ST} = 0.092, D = 0.077) than to inundation forest subpopulations (F_{ST} = 0.154 ± 0.045, D = 0.145 ± 0.047; Tables 31 & 41). The minor effects of recent spatial isolation as well as long-time ecological differentiation seem to intermingle.

Results of Fisher exact tests in order to compare the genotypic structure (Table 33) of the eleven subpopulations of *P. obliterata* reflect the pattern of genetic subdivision revealed by the fixation index F_{ST}, Nei's (1978) genetic distance D and the above-mentioned tests of genotypic discrimination between biotopes. Analogous tests to evaluate the allelic structure between subpopulations (Table 34) and biotopes (Table 36) are supposed to be more powerful (Goudet et al. 1996). They actually showed an even more pronounced differentiation, but follow the same trend as the former comparative parameters.

A cluster analysis of Nei's (1978) genetic distance D did not reveal any subpopulation groupings according to geographic position or biotope type (Fig. 53). Since the reliability of a dendrogram constructed from D is possibly biased by the high level of average heterozygosity in *P. obliterata* (Nei 1978), another cluster analysis was computed from Tomiuk and Loeschcke's (1991) genetic distance, which is based on genotype frequencies. It displayed a somewhat discrete status of the upland subpopulations EB and AM (Fig. 54).

These two plantation subpopulations most likely originate from the same ancestral Peruvian upland population. One cannot exclude that both sites are still connected by

ongoing gene flow due to human activities, but the relative genetic similarity of the upland subpopulations most likely reflects historical rather than ongoing gene exchange. Even though the behaviour and environment of the two ecotypic forms (upland versus inundation forests) are in marked contrast, the allozyme data did not support biotope specific adaptation of *P. obliterata* in Central Amazonia. This does not rule out a relation between biotope-adaptive biological traits and genetic determination, because the evolution of ecotypes or speciation can occur in the absence of genetic differentiation detectable at the allozyme level and may involve only one or very few genes (Nevo 1990; Eber et al. 1991; Zhivotovsky et al. 1994; Mutebi et al. 1998; Kondrashov & Kondrashov 1999; Mopper et al. 2000). The frequencies of selectively neutral alleles reveal nothing about adaptive changes (Hartl 1988; Palo et al. 2003), but can provide information on the pattern of interaction between historically differentiated populations (Hilbish 1996). However, is has also been suggested that the degree of genetic differentiation in neutral markers may be closely predictive of the degree in loci coding quantitative traits (Merilä & Crnokrak 2001; McKay & Latta 2002). Future work should include additional subpopulations as well as the use of alternative markers to fully understand the processes of local acclimatisation and differentiation in the species.

4.3.1.4 Local Adaptation versus Phenotypic Plasticity

The *P. obliterata* subpopulations from upland and flooded environments seem to derive from a single population, this being consistent with the postulated origin in the Peruvian Andes (Golovatch & Sierwald 2001). Since the invasion of inundation forests is thought to have been a secondary event (Adis 1997), two discrete Peruvian populations may have been established in the respective biotopes. Whereas passive dispersal via waterways promotes the exchange of migrants within the inundation forests, overland gene flow among subpopulations from flooded and upland areas is evidently more restricted with regard to geographic distance. A limited gene flow across the ecologically dissimilar biotopes could have facilitated local adaptation by allowing microevolutionary changes that increased the fitness of resident populations, while it could maintain the observed genetic variation (Slatkin 1985). Further reduction in gene flow may result, where dispersing individuals experience low survival in the other biotope because they possess locally inappropriate traits (McPeek & Holt 1992). Such an ecological discrimination could hold true for immigrants from upland areas, which

may not cope equally well with seasonally flooded environments, while individuals from floodplain forests should thrive at non-flooded sites, as the absence of an aquatic phase facilitates survival and reproduction (cf. Chapter 3.3). Reduced hybrid fitness in the flooded biotope is possible if adaptations necessary for survival in this biotope are genetically fixed. In the event of local ecological selection (Schluter 2001), the observed level of gene flow maintained between opposed biotopes (EB and nearby inundation forests) seems only unidirectional, i.e. from inundation forests to upland areas. Nevertheless, ecologically driven speciation can also occur in the face of gene flow (Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999; Doebeli & Dieckmann 2003), with some loci undergoing divergent evolution, while others experience the homogenising effect of genetic exchange (Shaw & Danley 2003). The requirement of local survival strategies in the seasonally flooded biotopes could have induced disruptive selection and resulted in two coexisting phenotypic clusters (Dieckmann & Doebeli 1999), i.e. the observed ecotypes in *P. obliterata*. The process of adaptation to environmental conditions via phenotypic variants to genetically fixed criteria is known as 'genetic assimilation' (Waddington 1969). In its course, originally 'acquired characters' can become converted, by selection acting for several or many generations on the population concerned, into 'inherited characters' (Waddington 1975). The same could hold true for specific ecological features, e.g. a univoltine life cycle (Chapter 3.3.2.3.1), in the inundation forest populations of *P. obliterata*.

Alternatively, as suggested by comparative experiments (Chapter 3.2.5), the different life cycle strategies in *P. obliterata* may not result from local genetic adaptations but rather represent a response of populations through phenotypic plasticity of individuals to different ecological terms (cf. West-Eberhard 1989). In other words, ecological adaptation in *P. obliterata* may have been accomplished by environmentally induced phenotypic variability within a single genotype (cf. Parker et al. 1977). The postulated genetic basis for such phenotypic plasticity (genotype-environment interactions) is an epistatic gene regulation of structural genes, whose expression results in varying effects on the phenotype (Scheiner & Lyman 1991; Via et al. 1995). In this epistatic model, regulatory loci may cause other genes to be turned on or off in particular environments, involving both graded and switched responses (Via et al. 1995).

Metapopulation theory predicts a tension between the selection for good residents, which are locally adapted, and good dispersers, which are generally adapted (Mopper 1996). Long-distance dispersers have better opportunities to colonise novel

environments (Shaw 1995), as here observed for *P. obliterata*. Therefore, high colonisation ability is often associated with fixed 'general purpose' genotypes (GPG), i.e. generalists characterised by high phenotypic plasticity (Baker 1965, 1974; Owens et al. 1999) compared to the less flexible, native specialists (Parker et al. 1977; Kaufman & Smouse 2001). The GPG hypothesis proposes that selection occurring prior to colonisation may favour genotypes providing sufficient phenotypic 'responsiveness' to follow fluctuations in the environment (Parker et al. 1977; Boyce & Daley 1980). Such a flexible generalist strategy in the context of high geographical success in establishing colonies has already been suggested for thelytokous populations of the European millipede *Nemasoma varicorne* (Jensen et al. 2002). Furthermore, a positive relation between the molecular variance in populations and their phenotypic plasticity has been suggested (Fischer et al. 2000; Pluess & Stöcklin 2004), and *P. obliterata* appears both ecologically plastic (Chapter 3.2.5) and genetically diverse (Chapter 4.3.2). Sexton et al. (2002) suggested that both plasticity and adaptive evolution can contribute to the invasive potential of species. It is known that invasion events are often followed by rapid adaptive evolution in populations colonising new environments (Losos et al. 1997; Reznick et al. 1997). To invade successfully and survive environmental changes, colonising species such as *P. obliterata* may also need to possess appropriate genetic variation in traits most likely to be adaptive in the new environment (cf. Pease et al. 1989; Eriksson et al. 1993; Caroll & Dingle 1996; Petit et al. 2004). The genetically diverse *P. obliterata* could possibly meet this demand. Although molecular measures of genetic diversity often show only a limited ability to predict the variability of quantitative trait loci (QTLs) (Pfrender et al. 2000; Morgan et al. 2001; Reed & Frankham 2001), a correlation between heterozygosity at marker loci and heterosis could be observed (Charcosset & Essioux 1994). In addition, a correlation between genetic variation in neutral marker loci and QTLs at the multitrait scale has recently been reported (Zhan et al. 2005). Consequently, it has been suggested that simple genetic markers may be used to draw inferences about the amount of allelic variation (evolutionary potential) at QTLs (McKay & Latta 2002; Latta 2004).

The Amazonian millipede *Pycnotropis tida*, which is also widely distributed along the river Amazon/Solimões (Bachmann et al. 1998; Golovatch et al. 1998), comprises populations that are genetically much less diverse than those of *P. obliterata*, yet highly differentiated. *P. tida* populations on flooded and non-flooded sites are possibly genetically adapted to their respective biotopes. This would be consistent with the

observation that the species *P. tida*, in comparison with the eurytopic *P. obliterata*, is restricted to Várzea, mixedwater and secondary upland forests, but not found in Igapó, in primary upland forests or on plantations (Bachmann et al. 1998). Populations of the likewise genetically less diverse European millipede *Glomeris hexasticha* also reveal higher levels of differentiation (Hoess et al. 1997) than those of *P. obliterata* and appear to be more restricted in terms of their biotope range (Voigtländer & Düker 2001). The Australian millipede *Stygiochiropus communis*, which is endemic to caves, displays considerable genetic differentiation at geographic distances up to 61 km. Though *S. communis* occurs in quite varied cave habitats, it is restricted to an area of approximately 770 km^2 and not found in other, open biotope types (Humphreys & Shear 1993; Humphreys & Adams 2001) Therefore, it shows a minor geographic and biotope range compared to the eurytopic *P. obliterata*, apparently representing a stenotopic, most likely even stenoecious species. Genetic variability in the East Mediterranean *Tetrarthrosoma syriacum* is lower than in *P. obliterata*, but still high compared to some other millipedes (Pavlíček & Nevo 1996). Since only a small part of the species' range was covered in the study, it is difficult to compare genetic divergence in both millipedes. Low but distinct differentiation at a microgeographic level was found in the *T. syriacum* populations on two different slopes, which were only 200 m apart at the bottom, but varied considerably in microclimate, flora and fauna. The results suggested a more insular-like structure for the cooler, wetter, north-facing slope, where animals were far scarcer. According to the authors, the divergence between the slopes may correspond to the niche-width variation hypothesis, which predicts a positive correlation between niche-width and phenotypic (Van Valen 1965) or genotypic (Soule & Stewart 1970; Nevo 1990) variation.

Comparing the distribution, biotope range, population subdivision and genetic diversity of different millipedes, it appears that the species *P. obliterata* features a generalist strategy. The results show low divergence between populations of the diverse and widespread species, which can apparently cope well with abiotically varying biotopes (Chapter 3) and thus was able to invade seasonal inundation forests. Its colonising populations have possibly been selected to be flexible in the face of temporal variation in the environment and/or more tolerant to harsh environmental conditions, just as postulated for the thelytokous *N. varicorne* (Jensen et al. 2002).

4.3.2 Allozyme Variability Within Populations of *P. obliterata*

Pronounced genetic diversity was found within eleven subpopulations of *P. obliterata*. All loci under study, PGI, PGM, ME, GOT, PK and ACP, were polymorphic in each of the subpopulations (95 % criterion; Sperlich 1988). The mean number of alleles per locus, A = 3.5 (Table 24), as well as the mean ratio of actual (k) and effective (k_e) number of alleles per locus, k/k_e = 1.8 (Table 27), coincided almost completely in all biotopes. The mean number of alleles per locus in *P. obliterata* was greater than the average values in other millipedes: $A < 2$ in *Pycnotropis tida* (Bachmann et al. 1998; Golovatch et al. 1998), $1.3 \leq A \geq 1.8$ and $2.4 \leq A \geq 2.9$, respectively, in two *Glomeris* taxa, *G. intermedia* and *G. hexasticha* (Hoess et al. 1997), and A = 3.1 in *Tetrarthrosoma syriacum* (Pavlíček & Nevo 1996). This shows that the species *P. obliterata* exhibits high genetic variability (El Mousadik & Petit 1996). In addition, the mean level of heterozygosity in *P. obliterata*, H = 0.393 (Table 24), was relatively high (cf. Nobrega et al. 2004) compared to other millipedes: H = 0.096 in *P. tida* (Bachmann et al. 1998) and H = 0.233 in *T. syriacum* (Pavlíček & Nevo 1996).

Genetic variance seems to be largely determined by ecological rather than demographic or life history factors (Nevo 1990, 2001). The relationship between genetic variability at selectively neutral (or nearly neutral) loci and the geographic range of a species is not always obvious (Krafsur 1999). Allozyme diversity is partly correlated to, and predictable by, ecological heterogeneity. Species living in broader environmental spectra are generally characterised by higher genetic diversity than their counterparts (Nevo 1990, 2001). It is suggested that the increased genotypic variance documented in extreme environments may serve as a source of novel adaptations under changed environments (Badyaev 2005). Associations between the molecular genetic variability and the survival probability of a species over a wide range of environmental conditions have been reported, e.g. for tadpoles (Lesbarreres et al. 2005). Furthermore, spatial heterogeneity of the environment coupled with balancing selection due to biotope heterogeneity is considered to be an important mechanism maintaining such genetic variation (Nevo 1990; Spichtig & Kawecki 2004).

Hence, the above-mentioned varieties in genetic diversity may be consistent with different life strategies in the respective millipede species (Chapter 4.3.1.4). Along with the evident colonisation ability of *P. obliterata*, the low genetic divergence observed between Amazonian populations may correspond to a generalist strategy in this

millipede. The species' ecological plasticity could even buffer the existing genetic diversity from selection (Baker 1974; Schlichting 1986). In contrast, the less diverse populations of the Amazonian congener *P. tida* are evidently locally adapted and occupy a more restricted biotope range (Bachmann et al. 1998; Golovatch et al. 1998). The same seems to hold true for the eastern Mediterranean *T. syriacum* (Pavlíček & Nevo 1996) and also for the European *G. hexasticha*, which is frequent in mesoxeric grasslands, but absent from three other dry biotopes examined in eastern Germany (Voigtländer & Düker 2001).

The assumption that euryoecious species living in unstable, heterogeneous environments have high genetic variability (Hedrick et al. 1976; Sperlich 1988; Anlauf 1997) is supported by this study, since the genetically diverse *P. obliterata* has successfully colonised various biotopes, including seasonally flooded areas. Furthermore, its thelytokous populations have been found in European hothouses and even living openly in woodlands in the southern USA (Golovatch & Sierwald 2001; Shelley 2004). The comparatively high level of heterozygosity in the Amazonian populations of the species may be evidence of an increased fitness (Sperlich 1988; Mitton 1994). Considering genotype-environment interactions mediated by additive polygenic inheritance or epistasis, heterozygosity can also increase the robustness of the phenotype against environmental influences, leading to the maintenance of genetic variability even under selection (Gillespie & Turelli 1989; Gimelfarb 1989). Genetic diversity in turn appears to be relevant for the long-term viability of a species (El Mousadik & Petit 1996) and a prerequisite for future evolution (Eriksson et al. 1993), providing the basic material on which adaptation and speciation depend (Amos & Harwood 1998).

4.3.3 Synopsis

The study revealed high levels of diversity but low levels of differentiation among subpopulations in *P. obliterata*, thus supporting the classical opinion that in many cases most of the genetic variation resides within populations (El Mousadik & Petit 1996).

Even though the behaviour and environment of the populations on upland plantations and within inundation forests are in marked contrast, they lacked clear genetic differentiation. The allozyme data failed to support biotope-specific adaptations in *P. obliterata*. Whether any ecological divergence at specific loci exists or if the ability

to colonise such different environments is based upon general phenotypic flexibility remains to be clarified. However, the species appears to feature a generalist strategy. It seems plausible that the surveyed genetic diversity in *P. obliterata* is associated with the environmental heterogeneity within its range and represents a potential for future evolution.

The pronounced genetic variability, coupled with low differentiation in respect of geographic position or biotope type, are evidence for a complex and dynamic population history in *P. obliterata* in Central Amazonia. The genetic structure of the species seems to be shaped by the combining effects of long-time evolutionary and recent dispersal events. Historical incidents associated with the colonisation of inundation forests and upland plantations along with subsequent repeated downstream distribution along the rivers Solimões, Amazon and lower Negro apparently contributed to the observed genetic pattern. The relatively strong genetic cohesion of Amazonian populations of *P. obliterata* is therefore most likely explained by recent divergence and contemporary gene flow. That would also account for the failure to detect any pattern of isolation by distance, i.e. a relation between the geographic distribution and genetic similarity of subpopulations. Such a correlation was only observed at a larger scale, i.e. for the upland subpopulation at Amapá State which is located approximately 1,000 km east of Manaus. Furthermore, repeated dispersal events are in agreement with the inundation forest subpopulations to be treated as mixtures of resident and immigrant specimens, resulting in the observed lack of heterozygotes.

Dispersal supports in particular the exchange of genetic information between populations and the establishment of new ones, one of the most important phenomena for the survival of species (Gilpin & Soule 1986). The passive drift of invertebrates by rivers can thus be a key process in the recolonisation of dynamic floodplain biotopes (Tenzer & Plachter 2003). Apparently, multiple introductions along the rivers contribute to the observed lack of neutral divergence and replenish the substantial genetic diversity in *P. obliterata* during colonisation. This emphasises the importance of gene flow within the inundation forests and hence the relevance of undisturbed afforested river banks. Since biotopes along the river are arranged longitudinally, the chances for the transported individuals to find a suitable biotope are rather high (Tenzer & Plachter 2003).

5 CONCLUSIONS

Tropical forests are considered the most species-rich ecosystems on earth and harbour more than 50 % of all species (Schaller 2005). The high diversity of tropical organisms has intrigued biologists for centuries (e.g. Darwin 1839), but is not yet sufficiently understood, hence requiring a comprehension of the ecological and evolutionary dynamics for effective conservation strategies (Bermingham & Dick 2005). Beyond regional effects of climate and productivity (Turner et al. 1996; Gaston 2000; Whittaker et al. 2001), several additional hypotheses have been proposed to explain the high species richness in tropical forests. Most of these theories emphasise geographic isolation as the primary factor regulating speciation (cf. Simpson & Haffer 1978; Gascon et al. 2000). However, recent studies suggest that natural selection across ecological gradients may also give rise to speciation (Erwin & Adis 1982; Schneider et al. 1999; Basset et al. 2003). In particular the diversity of Neotropical arthropods is assumed to be based upon such processes in the Amazonian floodplain forests (cf. Adis 1997), but so far few studies have used an ecological and genetic approach to address this hypothesis (Wolf & Adis 1992; Bachmann et al. 1998). This thesis provides an example, where field data, genetic analysis and additional experimental data were combined to test for such an ecological speciation process in the eurytopic millipede *Poratia obliterata*.

The results presented in this thesis suggest that the development of survival strategies adaptive to annually flooded biotopes is not necessarily accompanied by speciation in local terrestrial arthropods. Field observations indicate ethological, phenological and physiological adaptations in *P. obliterata* from floodplain forests compared to specimens on non-flooded uplands (Chapter 3). When estimating the genetic variation however, no substantial evidence for biotope specific diversification was found (Chapter 4). For the first time it has been shown experimentally that alternative life strategies in specimens from floodplains represent phenotypic responses to adverse conditions that reverse after a return to favourable conditions (Chapter 3.2.5). The experiments provide evidence of a higher disposition, but not exclusiveness, for some adaptive responses found in animals from floodplain forests. In addition, some dispositions appear to be field-derived, with their transference to the next generation possibly mediated by maternal effects (Chapter 3.3.2.3.3). It seems that remarkable adaptive flexibility i.e. ecological plasticity enables *P. obliterata* to colonise the

contrasting biotopes (Chapter 4.3.1.4). The combined results from field, experimental and genetic studies in this thesis suggest that the occurrence of generalist fauna within the Amazonian floodplain forests might be greater than previously estimated (cf. Adis 1997).

A fundamental question raised by this study is how, and to what extent, plasticity contributes to high tropical diversity. It is still a matter of controversy whether phenotypic plasticity is a mechanism facilitating evolution. Some authors implicate plasticity in speciation (Pigliucci & Murren 2003; West-Eberhard 2003; Schlichting 2004), while de Jong (2004) opposed their conclusions and underlines the view of plasticity as an ecological adaptation (Via et al. 1995; Berrigan & Scheiner 2004). This thesis supports the latter view. Given that genotypes with higher levels of plasticity occupy wider niches (Soule & Stewart 1970; Nevo 1990) and are thus less affected by environmental change, plasticity might even delay speciation (de Jong 2004). Still, plasticity initially allows a species to colonise novel environments and thus may be a prerequisite for the adaptive evolution often following invasion events (cf. Owens et al. 1999; Sexton et al. 2002).

As the ecological variants of *P. obliterata* may easily be mistaken for discrete ecotypes (cf. Adis 1992b), this thesis further emphasises the need to employ genetic markers and/or other methods to understand and manage biodiversity. Extreme environments, such as the floodplains, can induce an increase in phenotypic and genotypic variance, and it is suggested that this variance is a source of novel adaptations under changed environments (Badyaev 2005). In this context, the substantial genetic diversity found in *P. obliterata* (Chapter 4.3.2) may allow for future evolution (cf. Eriksson et al. 1993) by providing the potential on which adaptation and speciation can occur (cf. Amos & Harwood 1998). These considerations highlight the requirement to preserve processes that promote genetic diversity in rainforest species (Basset et al. 2003). In the study area the main rivers apparently serve as dispersal routes for *P. obliterata* on islands and along river banks, with gene flow maintaining the species' high genetic diversity (Chapter 4.3.1.3). Detailed knowledge about the consequence of long-term isolation on the biological and genetic constitution of subpopulations is necessary to estimate the impact of restricted dispersal on *P. obliterata*. This may be explored through the investigation of genetic differentiation in isolated populations such as those found in European hothouses (Adis et al. 2001). Still, the importance of dispersal for biodiversity has long been recognised (Levins & Culver 1971; Hanski 1983; Tilman 1994; Hubbell

2001). Fauna in the Amazon Basin is mostly composed of subpopulations (Schaller 2005) connected via dispersal (as shown here for *P. obliterata*), with biotope fragmentation shown to be a serious threat to the local biota (Laurance et al. 2001). Over the next 20 years basin-wide patterns of forest fragmentation have been predicted on basis of current land use and development plans, involving hydroelectric dams and river canalisation projects that will particularly affect floodplain fauna (cf. Laurance et al. 2005). Bearing the former considerations in mind, this provides a challenge for conservation biologists, as impending changes in the Amazon Basin will have important consequences for dispersal and therefore the evolution of genetic structures in the local fauna.

6 SUMMARY

The periodic flood pulse of the Amazon River has been the main controlling factor in the local riverine ecosystem for at least two million years. The disturbance caused by the annual inundation is reflected in poorer species diversity in affected lowlands compared to upland areas. Seasonality of flooding, however, has enabled long-term acclimatisation in resident flora and fauna. Numerous adaptations, in some cases along with speciation, have evolved in local terrestrial invertebrates, such as *Poratia obliterata* (Kraus, 1960). This small millipede, which probably originates from the Andes, is currently known from a remarkably broad range of Central Amazonian biotopes, i.e. various seasonal inundation forests, upland forest and plantations. Like most native millipedes, *P. obliterata* appears to escape flooding by tree ascents. Such developed survival strategies adaptive to annual inundation can either reflect ecological plasticity or implicate ecological speciation, .i.e. 'biotope-specific races' or ecotypes. To assess the causal mode of adaptation, I combined ecological studies with genetic analyses.

To compare life history strategies of hypothesised ecotypes in non-flooded versus seasonally flooded biotopes, I surveyed the behaviour and phenology of *P. obliterata* populations on a local upland plantation and in a white-, black- and mixedwater inundation forest. In addition, I conducted comparative experiments with regard to migration potential and possible factors regulating reproduction. I found that millipedes in the inundation forests migrate in direct response to the rising and receding waters and take refuge on tree trunks during the aquatic phase. Experiments suggest that disposition for such vertical migrations is higher in animals native to these forests than in those originating from upland areas. In the former situation, animals then take shelter under bark, aggregate in groups close to the water line, probably avoiding dehydration, and repeatedly socialise with co-occurring millipede species of similar size. In contrast, specimens on the upland plantation dwell on the ground in moist decaying plant debris without aggregation or species interactions. This population shows a plurivoltine life cycle, i.e. continuous reproduction, whereas a univoltine life cycle, i.e. lack of offspring during flooding, seems characteristic for inundation forest populations. Here, oviposition is apparently restricted to the terrestrial phase after females returned to the ground. Experiments indicate an optional adult dormancy on tree trunks, but failed to show regulating effects of abiotic factors, such as habitat substrate, temperature,

humidity and resources. Both development and maturation in offspring were faster for populations from inundation forests compared to upland ones. This appears to be field-derived and may allow for the floodplain populations to cope with discontinuous reproduction due to inundation by a faster establishment of succeeding generations during the terrestrial phase.

Seasonal vertical migration, aggregation and a univoltine life cycle appear to be adaptive traits of individuals living in inundation forests. Specimens inhabiting flooded and non-flooded sites thus may be considered as different ecotypes. Therefore, I examined whether these ecological adaptations involve ecologically driven speciation processes, i.e. genetically differentiated populations. Eleven subpopulations of *P. obliterata* from ten different sites in Central Amazonia, including a non-flooded plantation and various inundation forests, and one upland locality in Amapá State were compared on the basis of six polymorphic enzyme loci. Even though the behaviour and environment of the ecotypic forms are in marked contrast, the allozyme data did not support biotope-specific genetic adaptations of *P. obliterata* in Central Amazonia. Genetic diversity in *P. obliterata* (A = 3.5; H = 0.393) is higher than the average value in millipedes, providing a potential for future evolution, while outcrossing rates appear to be high, since most of the genetic variation (ca. 90 %) resides within rather than among subpopulations (F_{ST} = 0.079). Hence, the alternative life strategies of populations in floodplain areas might be a phenotypic response to environmental constrains, suggesting ecological plasticity in this species. Estimates of gene flow indicate long-distance dispersal, probably downstream drift along the rivers, and/or recent expansion to the study area, on plantations likely due to introductions with plant material.

Comparing the distribution, biotope range, population subdivision and genetic diversity of different millipedes, the species *P. obliterata* appears to feature a generalist strategy. My results show low divergence between Amazonian populations of this diverse and widespread species, which seems to cope well with abiotically varying biotopes and thus successfully invaded seasonal inundation forests.

7 ZUSAMMENFASSUNG

Seit über 2 Millionen Jahren ist der jahresperiodische Flutpuls des Amazonas die primäre Steuergröße für die angrenzenden Überschwemmungsgebiete. Die Artenvielfalt auf den betroffenen Landflächen ist zwar geringer als die in höher gelegenen Regionen, die Saisonalität der Überflutung ermöglichte jedoch die langfristige Anpassung der ansässigen Flora und Fauna. Bei den terrestrischen Arthropoden sind zahlreiche Adaptationen bekannt, die in einigen Fällen zur Artbildung führten. Die Tausendfüßerart *Poratia obliterata* (Kraus, 1960) besiedelt gegenwärtig ein breites Spektrum von zentralamazonischen Biotopen, zu denen sowohl diverse saisonale Überschwemmungswälder als auch Festlandwald und Plantagen zählen. Wie die meisten Diplopoden im Überschwemmungswald scheint *P. obliterata* dem Hochwasser durch das Emporklettern an Bäumen auszuweichen. Derartige Überlebensstrategien können ihren Ursprung entweder in ökologischer Plastizität oder in ökologischen Artbildungsprozessen, z. B. der Ausbildung von Ökotypen oder „biotop-spezifischen Rassen", haben. In der vorliegenden Arbeit wurden daher ökologische Untersuchungen in Freiland und Labor mit genetischen Analysen kombiniert, um den zugrunde liegenden Adaptationsmodus bei *P. obliterata* aufzudecken.

Um die Lebensstrategien potentieller Ökotypen zu vergleichen, wurden das Verhalten und die Phänologie von *P. obliterata* Populationen auf einer lokalen Festlandplantage und in je einem saisonalen Weiß-, Schwarz- und Mischwasserüberschwemmungswald untersucht. Ergänzende Experimente sollten Auskunft über das jeweilige Migrationspotential sowie eine möglicherweise abiotische Regulation der Fortpflanzung geben.

Die Tiere in den Überschwemmungswäldern weichen dem auflaufenden Wasser temporär in die Stammregion aus und kehren bei sinkendem Wasserpegel auf den Waldboden zurück. Die Neigung zur Vertikalwanderung ist bei den ansässigen Tieren stärker ausgeprägt als bei Tieren, die aus der Festlandregion stammen. Während der Überflutungsphase verbergen sich die Tausenfüßer unter der Baumrinde und bilden Gruppen - auch in Vergesellschaftung mit anderen Diplopodenarten - in der Nähe der Wasserlinie, vermutlich um Austrocknung zu vermeiden. Dagegen halten sich die Tiere der Festlandplantage in feuchtem Pflanzenmaterial auf dem Boden auf und zeigen weder Aggregation noch Interaktionen mit anderen Arten. Auch der Lebenszyklus unterscheidet sich. Während die Festlandpopulation einen plurivoltinen Lebenszyklus (kontinuierliche Reproduktion) aufweist, scheint ein univoltiner Lebenszyklus (ohne

Nachwuchs während der Überflutungsphase) kennzeichnend für Populationen in Überschwemmungswäldern zu sein. Experimente deuten eine optionale Dormanz der adulten Tiere in der Stammregion an, wobei eine Regulation durch abiotische Faktoren (Habitatsubstrat, Temperatur, Feuchtigkeit und Nahrungsressourcen) nicht nachgewiesen werden konnte. Entwicklung und Geschlechtsreife erfolgen bei dem Nachwuchs der Überschwemmungswaldpopulation vergleichsweise schneller. Dies wird offenbar durch Umwelteinflüsse bewirkt und erlaubt den betroffenen Tieren vermutlich die Etablierung von zwei aufeinander folgenden Generationen in der terrestrischen Phase.
Da saisonale Wanderungen, Aggregationen und ein univoltiner Lebenszyklus adaptive Merkmale der Individuen in Überschwemmungswäldern zu sein scheinen, könnten die Populationen in den verschiedenen Biotopen als Ökotypen angesehen werden. Um zu untersuchen, ob diese ökologischen Anpassungen von *P. obliterata* mit Artbildung einhergehen, wurden Subpopulationen von zehn zentralamazonischen Standorten (eine Festlandplantage und diverse Überschwemmungswälder) und einer Plantage aus dem ostamazonischen Amapá anhand von sechs polymorphen Enzymloci verglichen. Obwohl sich das Verhalten und die Umwelt der ökologischen Varianten stark unterscheiden, belegen die Allozymdaten keine biotopspezifische Differenzierung der Populationen. Die alternativen Lebensstrategien der Tiere in den Überschwemmungswäldern stellen daher vermutlich eine phänotypische Reaktion auf Umwelteinschränkungen dar, die auf ökologische Plastizität hindeutet. Die genetische Diversität von *P. obliterata* (A = 3,5; H = 0,393) ist zudem höher als der Durchschnittswert bisher untersuchter Tausendfüßerarten und könnte Potential für zukünftige Evolution bieten. Da der Großteil dieser Variabilität (ca. 90 %) innerhalb anstatt zwischen den Subpopulationen (F_{ST} = 0,079) zu finden ist, scheinen die Auskreuzungsraten hoch zu sein. Abschätzungen des Genflusses weisen auf Ausbreitung über weite Distanzen hin, vermutlich durch passive Verdriftung flussabwärts, und/oder auf eine erst kürzlich erfolgte Ansiedlung der Art im Untersuchungsgebiet.
Vergleicht man die Verbreitung, das Biotopspektrum, und die genetische Differenzierung und Diversität verschiedener Tausendfüßerarten, so scheint *P. obliterata* eine Generalisten-Strategie zu verfolgen. Die Ergebnisse meiner Untersuchungen zeigen eine geringe Divergenz zwischen den amazonischen Populationen dieser genetisch diversen, weit verbreiteten Art, die offenbar mit abiotisch variierenden Biotopen gut zurecht kommt und daher in der Lage war, die Überschwemmungswälder zu besiedeln.

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12 APPENDIX

Table 30a. Single locus (PGI) estimates of the average pairwise differences among the studied subpopulations of *P. obliterata.* All values are highly significant ($P < 0.000$). Above diagonal: average number of pairwise differences between subpopulations ($P_i$xy); diagonal elements (bold): average number of pairwise differences within a subpopulation (P_ix); below diagonal: corrected average pairwise difference ($P_i$xy-(P_ix+P_iy)/2).

	JC	**IP**	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	**0.824**	0.918	0.867	0.906	0.877	0.940	0.868	0.902	0.917	0.865	0.864
IP	0.098	**0.815**	0.862	0.806	0.830	0.778	0.845	0.818	0.847	0.833	0.840
MA	0.019	0.019	**0.871**	0.852	0.843	0.849	0.854	0.854	0.868	0.840	0.855
IC	0.122	0.027	0.044	**0.745**	0.779	0.810	0.810	0.774	0.774	0.770	0.876
PG	0.051	0.008	-0.007	-0.008	**0.830**	0.825	0.827	0.808	0.824	0.793	0.859
AV	0.150	-0.008	0.035	0.059	0.032	**0.757**	0.861	0.804	0.849	0.827	0.835
TM	0.032	0.014	-0.006	0.013	-0.012	0.058	**0.848**	0.845	0.837	0.825	0.864
PQ	0.080	-0.000	0.009	-0.009	-0.017	0.016	0.011	**0.820**	0.816	0.798	0.865
LJ	0.100	0.034	0.0270	-0.004	0.003	0.065	0.007	0.001	**0.811**	0.824	0.903
EB	0.052	0.024	0.003	-0.004	-0.023	0.047	-0.000	-0.013	0.017	**0.802**	0.858
AM	0.024	0.004	-0.009	0.075	0.0160	0.028	0.011	0.027	0.069	0.029	**0.857**

Table 30b. Single locus (PGM) estimates of the average pairwise differences among the studied subpopulations of *P. obliterata.* All values are highly significant ($P < 0.000$). Above diagonal: average number of pairwise differences between subpopulations ($P_i$xy); diagonal elements (bold): average number of pairwise differences within a subpopulation (P_ix); below diagonal: corrected average pairwise difference ($P_i$xy-(P_ix+P_iy)/2).

	JC	**IP**	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	**0.374**	0.488	0.566	0.455	0.609	0.771	0.567	0.391	0.534	0.510	0.694
IP	0.010	**0.581**	0.609	0.555	0.666	0.791	0.632	0.506	0.603	0.601	0.762
MA	0.059	-0.002	**0.641**	0.616	0.695	0.785	0.670	0.576	0.642	0.651	0.781
IC	-0.003	-0.006	0.025	**0.542**	0.647	0.765	0.620	0.477	0.592	0.564	0.693
PG	0.055	0.009	0.008	0.010	**0.733**	0.749	0.700	0.619	0.681	0.656	0.687
AV	0.256	0.171	0.136	0.166	0.054	**0.657**	0.768	0.768	0.763	0.712	0.531
TM	0.041	0.003	0.011	0.010	-0.006	0.101	**0.677**	0.581	0.649	0.644	0.728
PQ	-0.012	-0.001	0.039	-0.010	0.037	0.223	0.026	**0.432**	0.550	0.528	0.697
LJ	0.025	-0.010	-0.002	-0.002	-0.009	0.112	-0.012	0.011	**0.646**	0.621	0.721
EB	0.029	0.017	0.037	-0.001	-0.004	0.090	0.011	0.018	0.004	**0.588**	0.610
AM	0.346	0.310	0.299	0.261	0.159	0.041	0.228	0.319	0.237	0.154	**0.323**

Table 30c. Single locus (ME) estimates of the average pairwise differences among the studied subpopulations of *P. obliterata.* All values are highly significant ($P < 0.000$). Above diagonal: average number of pairwise differences between subpopulations ($P_i$xy); diagonal elements (bold): average number of pairwise differences within a subpopulation (P_ix); below diagonal: corrected average pairwise difference ($P_i$xy-(P_ix+P_iy)/2).

	JC	**IP**	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	**0.192**	0.165	0.334	0.156	0.213	0.158	0.264	0.172	0.258	0.244	0.277
IP	0.003	**0.133**	0.295	0.131	0.191	0.127	0.225	0.135	0.222	0.208	0.240
MA	0.030	0.020	**0.417**	0.307	0.350	0.299	0.359	0.299	0.361	0.351	0.371
IC	-0.004	0.000	0.034	**0.129**	0.184	0.125	0.233	0.138	0.228	0.213	0.247
PG	-0.006	0.001	0.018	-0.003	**0.246**	0.185	0.283	0.197	0.279	0.266	0.297
AV	-0.002	-0.003	0.027	-0.003	-0.001	**0.127**	0.227	0.134	0.222	0.208	0.241
TM	0.010	0.001	-0.007	0.012	0.003	0.006	**0.315**	0.231	0.303	0.291	0.317
PQ	0.003	-0.005	0.017	0.000	0.001	-0.003	-0.000	**0.148**	0.228	0.214	0.246
LJ	0.007	0.001	-0.003	0.008	0.001	0.004	-0.010	-0.001	**0.310**	0.288	0.315
EB	0.005	-0.001	0.000	0.006	0.001	0.002	-0.008	-0.002	-0.010	**0.284**	0.303
AM	0.011	0.004	0.007	0.013	0.004	0.008	-0.010	0.002	-0.010	-0.009	**0.340**

Table 30d. Single locus (GOT) estimates of the average pairwise differences among the studied subpopulations of *P. obliterata.* All values are highly significant ($P < 0.000$). Above diagonal: average number of pairwise differences between subpopulations ($P_i$xy); diagonal elements (bold): average number of pairwise differences within a subpopulation (P_ix); below diagonal: corrected average pairwise difference ($P_i$xy-(P_ix+P_iy)/2).

	JC	**IP**	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	**0.324**	0.413	0.263	0.282	0.426	0.296	0.211	0.259	0.376	0.270	0.190
IP	0.023	**0.456**	0.366	0.386	0.536	0.389	0.312	0.349	0.484	0.374	0.290
MA	-0.006	0.031	**0.214**	0.231	0.409	0.245	0.143	0.198	0.347	0.216	0.118
IC	-0.011	0.027	-0.007	**0.262**	0.416	0.266	0.172	0.223	0.359	0.239	0.148
PG	0.017	0.061	0.054	0.038	**0.495**	0.432	0.389	0.417	0.452	0.412	0.381
AV	-0.010	0.017	-0.006	-0.009	0.040	**0.288**	0.185	0.235	0.374	0.253	0.161
TM	0.016	0.051	0.003	0.007	0.108	0.008	**0.067**	0.128	0.313	0.154	0.033
PQ	0.001	0.0261	-0.004	-0.003	0.074	-0.004	-0.001	**0.191**	0.350	0.208	0.100
LJ	-0.003	0.039	0.023	0.011	-0.012	0.013	0.063	0.037	**0.434**	0.352	0.300
EB	-0.008	0.029	-0.007	-0.008	0.048	-0.007	0.004	-0.004	0.018	**0.232**	0.129
AM	0.029	0.062	0.011	0.017	0.133	0.017	0.000	0.005	0.083	0.013	**0.000**

Table 30e. Single locus (PK) estimates of the average pairwise differences among the studied subpopulations of *P. obliterata.* All values are highly significant ($P < 0.000$). Above diagonal: average number of pairwise differences between subpopulations ($P_i$xy); diagonal elements (bold): average number of pairwise differences within a subpopulation (P_ix); below diagonal: corrected average pairwise difference ($P_i$xy-(P_ix+P_iy)/2).

	JC	**IP**	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	**0.331**	0.452	0.600	0.460	0.580	0.366	0.607	0.316	0.448	0.564	0.593
IP	0.034	**0.503**	0.527	0.489	0.522	0.464	0.529	0.451	0.486	0.517	0.525
MA	0.204	0.045	**0.462**	0.522	0.456	0.575	0.440	0.602	0.529	0.465	0.448
IC	0.040	-0.016	0.037	**0.508**	0.518	0.470	0.524	0.459	0.489	0.514	0.521
PG	0.174	0.030	-0.015	0.024	**0.480**	0.560	0.452	0.582	0.523	0.472	0.459
AV	-0.007	0.005	0.137	0.009	0.113	**0.414**	0.580	0.363	0.461	0.548	0.570
TM	0.215	0.051	-0.016	0.044	-0.014	0.147	**0.452**	0.609	0.531	0.462	0.445
PQ	-0.011	0.038	0.210	0.044	0.180	-0.006	0.222	**0.323**	0.447	0.565	0.595
LJ	0.032	-0.017	0.047	-0.017	0.032	0.003	0.053	0.035	**0.502**	0.518	0.527
EB	0.152	0.019	-0.012	0.014	-0.015	0.094	-0.010	0.158	0.021	**0.492**	0.468
AM	0.194	0.039	-0.016	0.033	-0.016	0.129	-0.016	0.200	0.042	-0.013	**0.468**

Table 30f. Single locus (ACP) estimates of the average pairwise differences among the studied subpopulations of *P. obliterata.* All values are highly significant ($P < 0.000$). Above diagonal: average number of pairwise differences between subpopulations ($P_i$xy); diagonal elements (bold): average number of pairwise differences within a subpopulation (P_ix); below diagonal: corrected average pairwise difference ($P_i$xy-(P_ix+P_iy)/2).

	JC	**IP**	**MA**	**IC**	**PG**	**AV**	**TM**	**PQ**	**LJ**	**EB**	**AM**
JC	**0.340**	0.387	0.320	0.380	0.317	0.324	0.333	0.310	0.371	0.336	0.506
IP	0.006	**0.423**	0.372	0.404	0.372	0.368	0.374	0.360	0.412	0.376	0.517
MA	-0.009	0.002	**0.317**	0.366	0.306	0.311	0.320	0.298	0.361	0.323	0.497
IC	0.004	-0.015	0.000	**0.414**	0.366	0.362	0.368	0.353	0.407	0.370	0.515
PG	-0.011	0.003	-0.010	0.001	**0.315**	0.309	0.318	0.296	0.359	0.321	0.498
AV	-0.008	-0.005	-0.009	-0.006	-0.010	**0.323**	0.319	0.298	0.362	0.322	0.495
TM	-0.005	-0.006	0.007	-0.007	-0.008	-0.011	**0.337**	0.307	0.369	0.330	0.498
PQ	-0.007	0.000	-0.009	-0.002	-0.010	-0.011	-0.009	**0.296**	0.351	0.310	0.490
LJ	-0.007	-0.008	0.006	-0.008	-0.007	-0.008	-0.008	-0.005	**0.417**	0.371	0.520
EB	-0.004	-0.006	-0.007	-0.007	-0.007	-0.010	-0.009	-0.008	-0.008	**0.341**	0.499
AM	0.036	0.005	0.038	0.007	0.040	0.033	0.029	0.042	0.011	0.028	**0.601**

13 CURRICULUM VITAE

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Publikationen

Bergholz, N. G. R., Adis, J. & Golovatch, S. I. (2004): New records of the millipede *Myrmecodesmus hastatus* (SCHUBART, 1945) in Amazonia of Brazil (Diplopoda: Polydesmida: Pyrgodesmidae). Amazoniana 18: 157-161.

Bergholz, N. G. R., Adis, J. & Golovatch, S. I. (2005): The millipede *Poratia insularis* (Kraus, 1960) new to the fauna of Brazil (Diplopoda: Polydesmida: Pyrgodesmidae). Arthropoda Selecta 13: 123-127.

Bergholz, N. G. R. (2006): The millipede *Cylindrodesmus hirsutus* (Pocock, 1889) new to the fauna of Costa Rica (Diplopoda: Polydesmida: Haplodesmidae). Arthropoda Selecta 14: 297-298.

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14 ERKLÄRUNG

Hiermit versichere ich, dass diese Abhandlung – abgesehen von der Beratung durch meine akademischen Lehrer – nach Inhalt und Form meine eigene Arbeit ist und dass ich keine anderen als die angegebenen Hilfsmittel und Quellen verwendet habe. Die Arbeit hat bisher weder ganz noch zum Teil an anderer Stelle im Rahmen eines Prüfungsverfahrens vorgelegen. Bisher wurden noch keine Teile dieser Arbeit als Manuskripte in Zeitschriften eingereicht oder veröffentlicht. Die Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis entstanden.

Plön, den 27. September 2006

Natalie G. R. Bergholz