



Cleaning wrasse species vary with respect to dependency on the mutualism and behavioural adaptations in interactions

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Interspecific mutualisms are an essential feature of life on earth, yet we know little about their evolution and stability. In many mutualisms several species are available as partners, raising questions about the similarity in function and behavioural repertoire depending on the partner species. Furthermore, variation between species in the quantity and quality of interactions resulting in variation in payoffs may allow us to infer the potential evolutionary origin of a multispecies mutualism complex. We addressed these issues in the marine cleaning mutualism, in which so-called ‘cleaners’ remove ectoparasites from so-called ‘client’ reef fish. We measured several parameters concerning the quantity and quality of cleaning interactions in six sympatric cleaner wrasse species. We found significant variation between cleaner species with respect to client diversity, the number of interactions with predatory clients, the duration of interactions, the frequency of client jolts as a correlate of ‘cheating’ by cleaners, and behaviours used for manipulation of client decisions. Exploratory correlations between cleaner species’ dependency and our variables of interest suggest that cleaning originated as a conflict-free by-product mutualism and evolved towards more sophisticated behaviours, including strategic behaviours for interactions with predators, cheating and manipulation specifically adapted to the client type.

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Interspecific mutualisms, that is, mutually beneficial interactions between individuals of different species, are an essential feature of life on earth (Bronstein 2001). Many mutualisms are characterized by low partner specificity (Bronstein 2003), where any particular species may interact with many potential partner species. This is true, for example, for many pollination mutualisms (Vazquez & Simberloff 2002), ants and partner species mutualisms (Pierce et al. 2002) or marine cleaning mutualisms (Côté 2000). Such diversity of potential partner species raises two related questions. First, it is of interest to understand whether species differ with respect to the quality of service (the benefits) they provide their partners. Second, one may ask whether in species providing the same service, variation in the quality of service relates to variation in the degree to which they express behaviour that is apparently adapted to the interactions. We call the sum of such apparently adapted traits a species’ strategic ability with respect to their mutualistic interactions. The first question has been addressed repeatedly. For example, ant species differ with respect to how numerously they defend and how aggressively

they behave towards third-party species when defending their partner species. Being more numerous and aggressive is typically more beneficial for their partners because of the resulting increased protection from predators (Ness et al. 2006). However, in ant–plant mutualisms, ant aggressiveness is not purely beneficial as aggressive ant species may keep pollinators away (Ness 2006). In leguminose plant–rhizobia mutualisms, it is known that bacteria lineages differ with respect to their ability to fix nitrogen (Burdon et al. 1999). As a final example, flowering plant species differ with respect to both nectar quantity and nectar composition, depending on which kinds of pollinators they aim to attract (Brandenburg et al. 2009).

The question whether species that engage in the same type of mutualistic behaviours (e.g. pollination, cleaning) differ in their strategic options has received far less attention. Only extreme cases in which former partner species evolved into being parasitic species have been studied in detail, including nectarless plants such as orchids (Schiestl 2005) and lycaenid butterfly species that manipulate their ant hosts with pheromones, thereby gaining access to worker food, eggs and larvae (Pierce et al. 2002). While comparisons of the strategic behaviour of mutualist partner species are lacking, it has been noted that species involved in mutualisms often appear to lack specific adaptations for their interspecific interactions (Bergstrom et al. 2003). How any adaptation might be linked with the degree of both dependency and specificity of partner species remains unclear (Bronstein 2003). Here, we provide for the first time data on

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six sympatric species of cleaner wrasses describing the course of interactions with client reef fish in order to make inferences about variation in adaptations in this marine cleaner–client mutualism. Of these species only *Labroides dimidiatus*, which is an obligate cleaner wrasse, has been studied in detail before. These cleaner wrasses remove more than 1000 ectoparasites per day from a large variety of ‘client’ reef fish, including piscivorous species (Grutter 1996). A conflict of interests exists as cleaners prefer client mucus (which they obtain by biting clients) over ectoparasites (Grutter & Bshary 2003). Clients therefore use several control mechanisms such as image scoring, partner switching and punishment to make cleaners feed against their preference (Bshary & Grutter 2005, 2006). While these control mechanisms reduce cheating they cannot entirely prevent it. A particular behaviour in *L. dimidiatus* is tactile stimulation, a touch of the client’s body (typically at the dorsal area) with their pelvic and pectoral fins. Tactile stimulation is used for preconflict management, to reconcile and also to manipulate client decisions (Bshary & Würth 2001; Grutter 2004; Cheney et al. 2008).

For our comparative approach we first asked whether facultative cleaner wrasses have a similar diversity of clients to *L. dimidiatus*. Furthermore, we were particularly interested in studying whether facultative cleaner wrasses show similar behaviours that function to manipulate client decisions (tactile stimulation) and how often they cheat in comparison to *L. dimidiatus*. Cheating (i.e. the cleaner bites the client) correlates well with client jolts in response to cleaner fish mouth contact in both *L. dimidiatus* and cleaning gobies (Bshary & Grutter 2002; Soares et al. 2008a). Therefore, cheating can be easily estimated by counting jolts. Finally, we investigated how clients responded after jolts. If the behaviour of clients is well adapted to the cleaners’ level of exploitation, we predicted that cleaners that cause higher jolt rates should be chased more frequently by clients.

We also investigated the potential origins of the marine cleaning mutualism. Several authors have hypothesized that mutualistic interspecific interactions might start out as a parasitism. When the host species subsequently evolves strategies for counterexploitation, this can result in a mutualism because of mutual exploitation with overall net benefits for both partner species (Frank 2003; Aanen & Hoekstra 2007). Alternatively, it has been suggested that by-product benefits provided by one species is the starting point for coevolution and mutual benefits (Bergstrom et al. 2003). Here, we assumed that the proportion of time a species engages in cleaning interactions to obtain food reflects the dependency of the species on the mutualism. We consider this assumption warranted because the proportion of time feeding on a certain type of food (or engaging in a certain type of interaction with other species) reflects specialization and the ecological niche of a species. Moreover, all our study species apparently specialize on small invertebrates for their diet (Randall 1983), which suggests that the nutritional value of food items eaten during cleaning interactions does not differ much from that of food items eaten outside cleaning interactions. Furthermore, we assume that low dependency reflects the ancient condition while high dependency is derived. Given this, we can infer the origin of cleaning mutualism by analysing whether cleaners’ dependency is correlated with the ecological and behavioural parameters involved in cleaning interactions. From negative correlations between cleaner dependency and more sophisticated strategic behaviours such as cheating and manipulation, we may infer that the quality of interactions probably evolved from parasitism towards mutualism. From positive correlations, we could infer that interactions between less dependent cleaners and clients correspond to a by-product mutualism while increasing dependency on the part of the cleaner favoured increasingly strategic behaviour, thereby also increasing the potential of conflicts with clients. Additionally, to understand whether or not the correlations resulted from independent evolutionary events, we

further applied general linear models accounting for phylogenetic constraints.

METHODS

Field observations took place from September to November 2006 at Mersa Bareika, Ras Mohammed National Park in Sinai, Egypt. The six species studied are members of the wrasse family Labridae. Only *L. dimidiatus* has been studied in detail while for the other five species (*Larabicus quadrilineatus*, *Bodianus anthioides*, *Pseudocheilinus hexataenia*, *Thalassoma klunzingeri* and *Thalassoma lunare*), two of which (*L. quadrilineatus* and *T. klunzingeri*) are endemic to the Red Sea, only anecdotes are available (Côté 2000). Only *L. dimidiatus* is an obligate cleaner, defined as a species in which individuals depend on cleaning interactions for their diet for their entire life. The other five species have been classified as facultative cleaner species in which only juveniles clean but also use alternative food sources (Côté 2000). As we were interested in cleaning interactions we studied only juveniles.

All study animals were located along 300 m of coastline and were chosen for convenient access by researchers. The depths of the cleaning stations varied from 1 to 15 m, the deepest being those of *B. anthioides*. Cleaning stations of individuals of the same species were typically more than 10 m apart and therefore independent from each other. Depending on habitat structure and depth of the station, the observers positioned themselves about 2–3 m from the station. Forty-one individuals were observed: six to eight individuals per species, depending on local abundance. Some individuals disappeared before data collection was finished; therefore fewer observations from these individuals were available. We chose only subjects that actually cleaned during preliminary observations to make sure that we would get data on cleaning interactions. Because of this choice criterion, our mean values of percentage of time spent cleaning are probably higher than the true population average for the facultative cleaner species (only every *L. dimidiatus* individual cleaned during the preliminary observations, while we discarded observations of individuals of the other species). The juveniles of all six study species are very stationary. Therefore, the individual identity was inferred from the location of the fish, in combination with size estimates and, if possible, individual characteristics such as particular spots or scars.

Behavioural observations were performed by L.B. and C.G. and were balanced throughout the day from 0800 to 1700 hours and between observers. Each observation session lasted 15 min and individual cleaners were not observed more than twice per day. Each individual was observed for a total of 4–6 h, yielding a total of 201 h of observation. An interaction started when the cleaner and its client engaged in physical contact. The following information was noted on a white plastic plate, immediately after an interaction had terminated: cleaner fish identity, client species according to Randall (1983), duration of the interaction, number of client jolts, client responses to jolts (none, fleeing, aggression) and occurrence of tactile stimulation. Owing to the writing procedure, it was possible that we missed some interactions. This sampling bias is conservative, as it tends to reduce the measured activity of dependent cleaners more than the measured activity of rarely cleaning individuals.

Data Analyses

We distinguished between three client categories: predatory clients, resident nonpredatory clients with access to the local cleaning station only (in this study some stations included several cleaners of different species, so their resident clients had access to several cleaners), and visiting nonpredatory clients with large territories/home ranges and hence access to several cleaning stations. In all

analyses we used individual cleaner fish as the basic unit for statistics. The values represent means for each cleaner and client species that had been observed interacting with the cleaner.

Kruskal–Wallis tests were used to test for differences between the cleaner species with respect to our variables of interest. Spearman rank correlations and phylogenetic regressions (described in the next section) were calculated to test whether there was a link between a cleaner species' dependency on cleaning and our variables of interest. For the analysis on client species composition, we counted the species that interacted with each cleaner. For the interactions with predatory clients, we calculated the percentage of interactions in which the client had been a predator of small fish. As client species composition turned out to be significantly different between cleaner species, this variation could have potentially confounded the results for the analyses of service quality. As only small resident client species are the typical clients for facultative cleaners, we only used these species to analyse service quality differences with Kruskal–Wallis tests and the correlations/phylogenetic regressions. In addition, we conducted a third type of analysis, in which we identified eight client species that had interacted with at least four cleaner species. For each of these species, we first calculated correlations between cleaner species dependency and the variables for the assessment of service quality, and then used the correlation coefficients for one-sample Wilcoxon tests to investigate whether there was a positive or negative link between dependency and service quality.

To test for client responses to jolts as a function of cleaner species identity, we calculated for each cleaner how frequently a jolt was followed by immediate client movement (terminating the interaction and chasing the cleaner). The mean values per client species per cleaner were used to calculate mean frequencies of responses and aggressive responses per cleaner.

The phylogenetic regression

To test for the link between a species' dependency on cleaning and our variables of interest and to account for the effect of phylogenetic constraints, we considered the level of sequence similarity in the ribosomal mtDNA regions (12s and 16s) of the cleaner species based on published gene sequences (Westneat & Alfaro 2005; GenBank accessions AY279579, AY279615, AY279619, AY279634, AY279651, AY279718, AY279722, AY279737, AY850801, AY850862, AY850864 and AY850866). With this information, we constructed an unrooted phylogenetic tree for the six species (Fig. 1), which was used for the phylogenetic regressions. When sequences were not available for a given species, we considered the accessions of its closest relative (i.e. *Thalassoma quinquevittatum* instead of *T. klunzingeri*, *Pseudochelinus octotaenia* instead of *P. hexataenia*, *Bodianus rufus* instead of *B. anthioides*). Sequences were initially aligned using ClustalW (Thompson et al. 1994) and subsequently manually adjusted using the similarity criterion (Morrison 2006). Alignment lengths were 815 bp and 490 bp for 12s and 16s, respectively. Single-gene unrooted trees were reconstructed using RAxML (Stamatakis 2006) after the best-fit model was determined using MrAIC (Nylander 2004; i.e. best-fit models were GTR + G for both regions using the Akaike criterion; Tavaré 1986). Since topologies obtained from both regions were identical, they were assembled with the 'Average Consensus Tree' supertree algorithm by minimizing the sum of squared distances between the consensus and the source trees (Nylander 2004) using CLANN (Creevey & McInerney 2005). This method allows us to take the average branch lengths into account and is particularly efficient when all taxa are shared by the different topologies (Buerki et al. 2011). We then performed phylogenetic linear regressions with generalized estimating equations (GEE) for which several independent variables can be used (Paradis & Claude 2002). This method takes into account phylogenetic distances in the treatment of the phylogenetic inertia of variables (Martins &

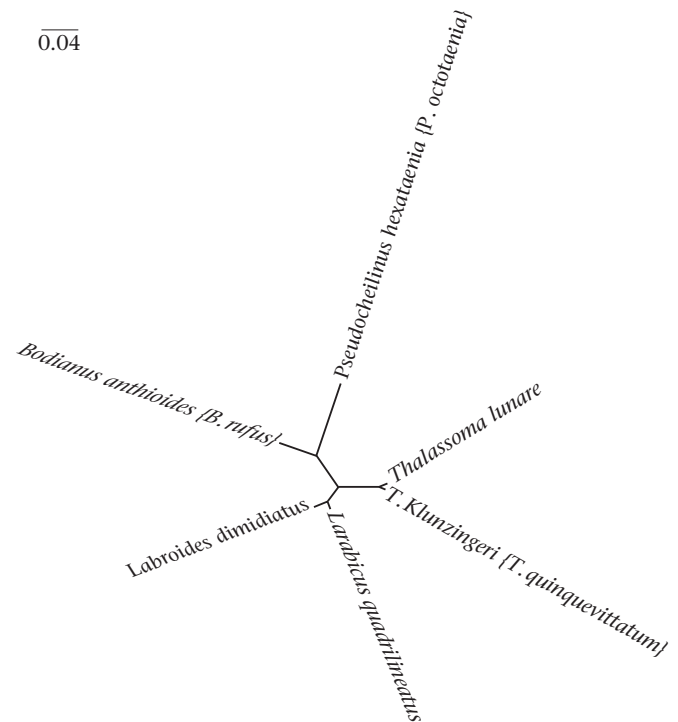


Figure 1. Unrooted phylogenetic tree of the six studied species. The tree is based on published gene sequences (Westneat & Alfaro 2005). For three species we did not have gene sequences and therefore used the information of a closely related species (names given in parentheses) instead.

Hansen 1997). We calculated a phylogenetic linear model of the dependency on cleaning against our variables of interest using the R CRAN APE package (Paradis et al. 2004). After normalizing the dependency variable using a neperian logarithm function, we addressed normality of model residuals with a Shapiro–Wilk W test ($P = 0.07$).

RESULTS

Variation in Dependency

When we used the percentage of time spent in interactions with clients as a measure of dependency of cleaners on cleaning interactions with respect to obtaining food, there was a significant difference in dependency between species (Kruskal–Wallis test: $\chi^2_5 = 27.1$, $N = 41$ cleaners, $P < 0.0001$; Fig. 2). Ranks of the six study species are as follows, from most dependent to least dependent: (1) *L. dimidiatus*, (2) *L. quadrilineatus*, (3) *B. anthioides*, (4) *P. hexataenia*, (5) *T. klunzingeri*, (6) *T. lunare*. Post hoc analyses showed that *L. dimidiatus* spent a significantly higher percentage of interactions with clients compared to all other species (Mann–Whitney U tests: all P values < 0.01). *Labricus quadrilineatus* also showed a significantly higher cleaner dependency than most facultative cleaner species (Mann–Whitney U tests: all P values < 0.01) but compared to *B. anthioides*, *L. quadrilineatus* only tended to show a higher cleaner dependency (Mann–Whitney U test: $Z = -1.86$, $P = 0.06$). There was no difference between all other facultative cleaner species (Mann–Whitney U tests: all P values > 0.18).

Variation in Client Composition

There was a significant difference between cleaner species with respect to the number of client species inspected (Kruskal–Wallis

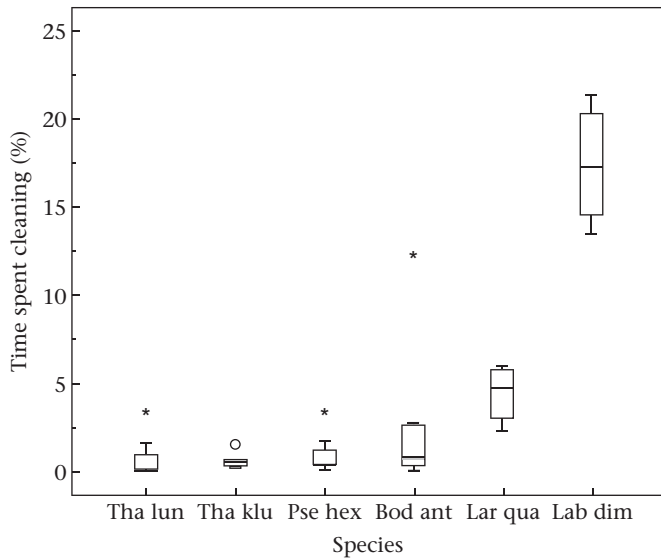


Figure 2. The percentage of time the six study species spent interacting with client species. Tha lun: *Thalassoma lunare*; Tha klu: *T. klunzingeri*; Pse hex: *Pseudocheilinus hexataenia*; Bod ant: *Bodianus anthioides*; Lar qua: *Larabicus quadrilineatus*; Lab dim: *Labroides dimidiatus*. The figure shows box plots with the median and interquartile ranges of mean values per individual cleaner. Vertical lines represent 1.5 times the interquartile range; circles are outliers and asterisks are extreme values.

test: $\chi^2_5 = 31.6$, $N = 41$, $P < 0.0001$; Fig. 3a) and with respect to the percentage of predatory clients among all interactions (Kruskal–Wallis test: $\chi^2_5 = 36.6$, $N = 41$, $P < 0.0001$; Fig. 3b). *Pseudocheilinus hexataenia* and the two *Thalassoma* species were never observed to clean predators and only one individual of *B. anthioides* cleaned a predator once. In contrast, *L. dimidiatus* and *L. quadrilineatus* regularly interacted with predators. To control for potential variation in local abundance of fish species, we also calculated the percentages of resident species cleaned relative to the number of species present at each station, and again found significant differences between the cleaner species (Kruskal–Wallis test: $\chi^2_5 = 36.6$, $N = 41$, $P < 0.0001$; Fig. 3c). *Labroides dimidiatus*, the most dependent cleaner species, interacted with the greatest number of client species, when we controlled for local diversity.

To test whether the differences in client diversity are merely a result of *L. dimidiatus* and *L. quadrilineatus* spending more time interacting with clients, we ran two additional analyses. First, we calculated a diversity index (mean number of species/(mean number of individuals/species cleaned)), which shows that *L. dimidiatus* cleaned the overall highest diversity of clients (mean \pm SE = 2.84 ± 0.24), followed by the other species (*L. quadrilineatus*: 2.49 ± 0.27 ; *B. anthioides*: 1.40 ± 0.31 ; *T. lunare*: 1.05 ± 0.31 ; *T. klunzingeri*: 0.89 ± 0.12 ; *P. hexataenia*: 0.49 ± 0.16). Second, we looked at client diversity as a function of the number of observation sessions. There was no indication that the low client diversity observed for facultative cleaners was due to fewer interactions per session. After 25% of observation sessions the mean percentage of total client species observed to interact with cleaners varied between 45% and 60%, and after 75% of observation sessions the diversity approached an asymptote, varying on average between 85% and 100% (Fig. 4). The values for *L. dimidiatus* were intermediate relative to the other species.

Variation in Service Quality

Focusing on small (smaller than 10 cm) resident nonpredatory clients, we found significant variation between cleaner species with respect to all three measured parameters of service quality

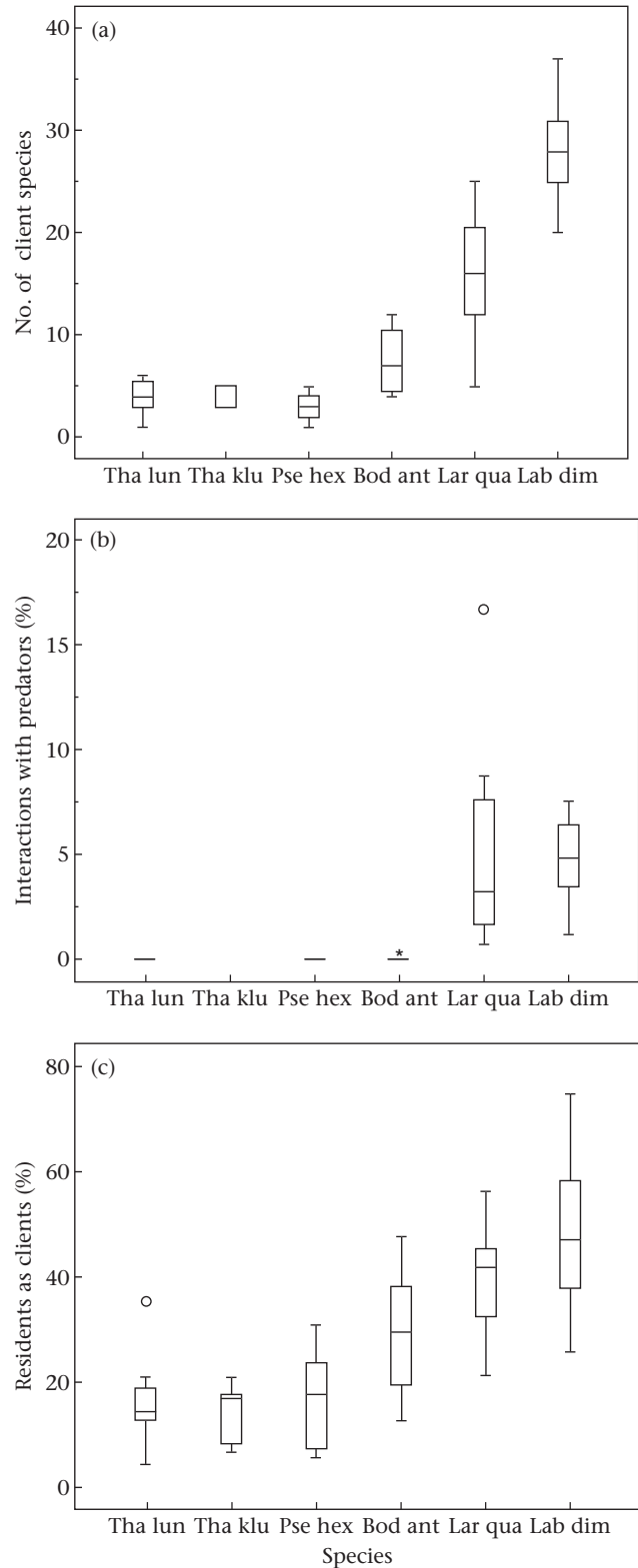


Figure 3. Three measures of client species composition for the six study species. Tha lun: *Thalassoma lunare*; Tha klu: *T. klunzingeri*; Pse hex: *Pseudocheilinus hexataenia*; Bod ant: *Bodianus anthioides*; Lar qua: *Larabicus quadrilineatus*; Lab dim: *Labroides dimidiatus*. (a) Total number of client species; (b) percentage of interactions in which the client was a predator of small fishes; (c) percentage of resident client species that were present at a cleaning station that was actually inspected. The figures show box plots with the median and interquartile ranges of mean values per individual cleaner. Vertical lines represent 1.5 times the interquartile range; circles are outliers.

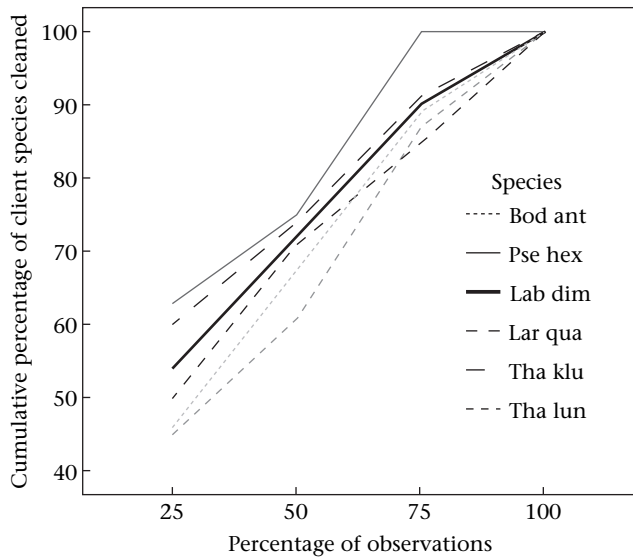


Figure 4. The percentage of client species cleaned as a function of the percentage of observation sessions. Tha lun: *Thalassoma lunare*; Tha klu: *T. klunzingeri*; Pse hex: *Pseudochelinus hexataenia*; Bod ant: *Bodianus anthioides*; Lar qua: *Larabicus quadrilineatus*; Lab dim: *Labroides dimidiatus*.

(Kruskal–Wallis tests: duration of interaction: $\chi^2_5 = 14.9$, $N = 41$, $P = 0.011$; jolt rate: $\chi^2_5 = 16.2$, $N = 41$, $P = 0.006$; tactile stimulation: $\chi^2_5 = 36.0$, $N = 41$, $P < 0.0001$; Fig. 5a–c). Only individuals of the two most dependent species regularly provided tactile stimulation to their clients, a behaviour that was never observed in *P. hexataenia* and *T. lunare*.

Variation in Client Responses to Jolts

There were significant differences between the six cleaner species concerning the probability that clients terminated the interaction after jolting (Kruskal–Wallis test: $\chi^2_5 = 11.7$, $N = 28$, $P = 0.04$; Fig. 5d). Moreover, there was a significant difference between the six species with respect to the percentage of aggressive client responses after jolting (Kruskal–Wallis test: $\chi^2_5 = 12.4$, $N = 22$, $P = 0.03$). The most dependent species, *L. dimidiatus* and *L. quadrilineatus*, received fewer aggressive responses per jolt than the other more facultative cleaner species.

Cleaner Dependency and Cleaning Interaction Parameters

Cleaner dependency was significantly positively correlated with client diversity (Spearman rank correlation: $r_s = 0.829$, $N = 6$, $P = 0.042$), the proportion of resident species present that were actually cleaned (Spearman rank correlation: $r_s = 0.943$, $N = 6$, $P = 0.005$) and the proportion of cleaning interactions with a predator (Spearman rank correlation: $r_s = 0.94$, $N = 6$, $P = 0.005$). The differences in client composition persisted when we applied sequential Bonferroni corrections (Rice 1989) to account for three tests (initial $\alpha' = 0.017$).

There was a significant positive relationship between cleaner dependency and the three measures of service quality that we used, for the eight most important client species (Wilcoxon tests: duration: $Z = -1.992$, $N = 8$, two ties, $P = 0.046$; client jolt rate: $Z = -2.380$, $N = 8$, $P = 0.017$; tactile stimulation: $Z = -2.524$, $N = 8$, $P = 0.012$; Table 1). The differences in our measures of cleaner behaviour persisted when we applied sequential Bonferroni corrections (Rice 1989) to account for three tests (initial $\alpha' = 0.017$). In contrast, there were

no significant correlations between dependency and client probability of terminating an interaction in response to a jolt (Spearman rank correlation: $r_s = 0.486$, $N = 6$, $P = 0.329$) or between dependency and the probability that clients would be aggressive after a jolt (Spearman rank correlation: $r_s = -0.725$, $N = 6$, $P = 0.103$).

Inferences from the Phylogenetic Regression

When phylogenetic regressions were performed, dependency on cleaning interaction showed a significant effect on three of our variables of interest: client diversity, percentage of interactions with predators and duration of interactions (Table 2). The ecological signal addressed for these three variables is thus not biased by a shared evolutionary history (potentially reflected in the phylogeny) among the species studied.

DISCUSSION

We had asked to what extent various sympatric cleaners of the family Labridae resemble each other in how they interact with client reef fishes, and whether any variation may correlate with variation in dependency on cleaning interaction for a cleaner's diet. We found significant variation between species with respect to all parameters of interest, documenting that cleaner–client interactions differ substantially depending on the cleaner species involved. The potential links between dependency and our parameters of interest are less straightforward.

Client Composition

The most dependent cleaner species, *L. dimidiatus*, interacted with a strikingly larger spectrum of client species, including predators, than the other less dependent species. The result was apparently not caused by larger sample sizes for *L. dimidiatus*, and the relative contributions of single client species were also more equilibrated in *L. dimidiatus*. This is in marked contrast to other mutualisms in which increased dependency of species typically goes along with an increase in partner specificity (or specialization): ant–lycaenids (Pierce et al. 2002), plant–seed-predating pollinators (Dufay & Anstett 2003; Molbo et al. 2003) and nutrition symbioses (Herre et al. 1999). In our study, high dependency seemed to involve adaptations that facilitated the interactions with predators, although the nature of the potential adaptations remains currently elusive. This conclusion differs from that drawn by Floeter et al. (2007) who, based on a literature search, did not find any link between dependency and predators as clients in a between-species comparison. The differing conclusions may be caused by the types of data sets used and the resulting enhanced variation owing to ecological differences between sites. Our study provides the first large data set from a single study site and hence reduces the potential problem of confounding ecological differences between sites. Because variations in client composition and in the percentage of interactions with predators are confirmed by the phylogenetic regression, these variables should be seen as fast evolving and not related to putative phylogenetic constraints.

Differences in Behaviour during Cleaning Interactions

With respect to service quality, only the two most dependent cleaner species, *L. dimidiatus* and *L. quadrilineatus*, consistently exhibited 'strategic' behaviour, that is, only individuals of those species consistently interacted with predators and regularly provided tactile stimulation. In addition, it appears that facultative cleaners cheated their clients less frequently than dependent cleaners. When we accounted for phylogenetic constraints, the duration of

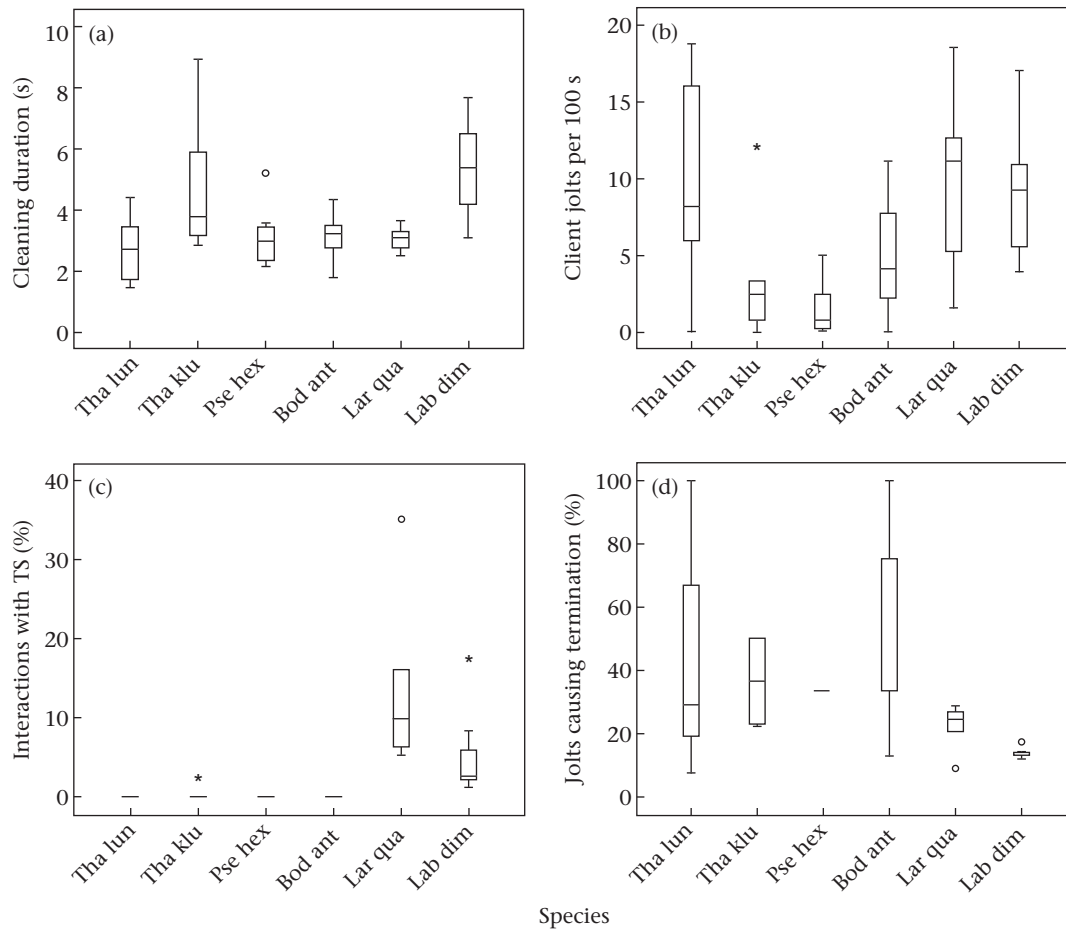


Figure 5. Four measures that describe the course of interactions for the six study species. Tha lun: *Thalassoma lunare*; Tha klu: *T. klunzingeri*; Pse hex: *Pseudocheilinus hexataenia*; Bod ant: *Bodianus anthioides*; Lar qua: *Larabicus quadrilineatus*; Lab dim: *Labroides dimidiatus*. (a) Duration of interactions (s); (b) client jolts per 100 s interaction; (c) percentage of interactions during which the cleaner provided tactile stimulation (TS) to its client; (d) percentage of jolts that were immediately followed by the client terminating the interaction. The figures show box plots with the median and interquartile ranges of mean values per individual cleaner. Vertical lines represent 1.5 times the interquartile range; circles are outliers and asterisks are extreme values.

interactions still remained significant: this trait might be a key feature in adaptation to different clients regardless of potential constraints reflected in the phylogeny. Our results on the four facultative cleaner species fit the common perception that mutualisms often lack obvious adaptations in the partner species (Bronstein 2001). Apparently, even Caribbean cleaning gobies of the genus *Elacatinus* lack strategic behaviour (Soares et al. 2008b), although they are highly dependent on cleaning interactions for their diet (Côté

2000). A potential explanation for a lack of strategic behaviour would be that the cleaner species in question prefer client ectoparasites over client mucus (see also Treasurer 2002). Under such circumstances, cleaners prefer to cooperate and the benefits to clients

Table 1
Analyses controlling for client species identity

Client species	N	Duration	Jolt frequency	Tactile stimulation
<i>Chromis dimidiata</i>	6	0.60	0.83	0.78
<i>Chromis ternatensis</i>	6	0.20	0.43	0.66
<i>Amblyglyphidodon leucogaster</i>	6	0.37	0.55	0.70
<i>Amblyglyphidodon flavilatus</i>	5	0.56	-0.05	0.71
<i>Anthias squamipinnis</i>	5	0.00	0.87	0.78
<i>Cirripectes castaneus</i>	5	0.00	0.56	0.78
<i>Ctenochaetus striatus</i>	4	-0.20	0.80	0.74
<i>Pomacentrus trichourus</i>	4	0.80	1.00	0.26

The table shows correlation coefficients (Spearman rank correlations) between cleaner species' dependency and three measures of service quality (duration, jolt frequency and occurrence of tactile stimulation) for the eight client species that were observed interacting with four, five or six different cleaner species (N).

Table 2
Analyses controlling for phylogenetic relationships between cleaners

Variable	Estimate	SE	ESP	t	Pr(T> t)
Client diversity	0.157	0.020	0.133	7.826	0.024*
% Resident species cleaned/resident species present	0.089	0.021	0.349	4.243	0.067
% Interactions with predators	0.621	0.103	0.214	6.011	0.038*
Duration of interactions (s)	0.537	0.084	0.217	6.396	0.034*
Client jolt rate	-0.135	0.266	3.626	-0.507	0.669
% Interactions with tactile stimulation	0.120	0.114	2.062	1.057	0.416
Probability of conflict after a jolt	0.047	0.078	2.260	0.608	0.614
Probability of aggressive response in conflicts	-0.011	0.011	2.141	-0.986	0.442

Phylogenetic linear regression with GEE of species dependency on cleaning against our variables of interest for the whole data set (41 observations among six species), and more specifically against client responses to jolts for all cleaners where jolts were observed (28 observations among six species). Values were averaged at the cleaner species level (N = 6; phylogenetic degrees of freedom = 3.717; for more information see Paradis & Claude 2002). ESP: estimated scale parameter; Pr(T>|t|): P value (for more information see Paradis et al. 2004). Only resident nonpredatory client species less than 10 cm in total length were considered.

*P < 0.05.

become a by-product of self-serving behaviour of cleaners. This scenario appears to fit the cleaning goby mutualism (Soares et al. 2011). In contrast, we know that *L. dimidiatus* prefers mucus over parasites (Grutter & Bshary 2003), which causes conflicts of interest between cleaner and client. Such a conflict should select for behaviours that allow the cleaners to shift the outcome of the interaction in their favour. Our results suggest that *L. quadrilineatus* (and *T. lunare*) also prefer mucus because of the high client jolt rates. Also, we provided the first description of tactile stimulation by a cleaner species outside of the genus *Labroides*. As the two genera *Labroides* and *Larabicus* appear to be sister taxa (Westneat & Alfaro 2005), it is possible that tactile stimulation evolved only once in a common ancestor of the two genera. Future research should conduct food choice tests on a variety of cleaner species to find out which species prefer mucus and which prefer ectoparasites. We would predict that our four more facultative study species that apparently lack strategic behaviour should prefer ectoparasites over mucus.

Client Behaviour

Clients regularly chased cleaners in response to jolts, but facultative cleaners were chased more often compared to *Labroides* or *Larabicus*. This could be because of the effects of tactile stimulation offered by the latter two species (Grutter 2004; Cheney et al. 2008). For instance, a study of *L. dimidiatus* suggested that these cleaners use tactile stimulation to manipulate client decisions over how long to stay for an inspection, and to reduce the likelihood that clients terminate the interaction in response to a bite from the cleaner (Bshary & Würth 2001). Moreover, cleaners provided more tactile stimulation to predatory clients, possibly as a means of preconflict management (Grutter 2004), with positive consequences even for potential prey (Cheney et al. 2008). We hypothesize that tactile stimulation is a key adaptation that facilitates increased exploitation of clients by cleaners.

In addition, adaptations in the jaw anatomy that have been described for the genus *Labroides* (Westneat et al. 2005) may allow them to remove parasites more efficiently and therefore clients may be more tolerant to bites. However, this possibility does not explain why clients are more often aggressive towards facultative cleaners. Aggression may yield benefits if it functions as punishment and hence improves levels of cooperation by the partner during future interactions (Clutton-Brock & Parker 1995). Punishment is thus aimed at resolving conflicts of interest. The key potential conflict of interest in cleaning mutualism is over what cleaners should eat on clients, and according to our current knowledge this conflict should be stronger in interactions involving *L. dimidiatus* than in interactions involving facultative cleaner species. Clients of Caribbean gobies do not show any aggression towards their cleaners (Soares et al. 2008b), and it appears that these cleaners prefer client ectoparasites over mucus (Soares et al., unpublished data), which would greatly reduce conflicts of interest between cleaners and clients. Thus, the possibility exists that the evolution of client behaviour towards cleaners is driven by the game structure of interactions with the most dependent cleaner species. In that scenario, fishes of the Indo-Pacific region would be adapted to interactions with *Labroides* cleaners and hence to the conflict of interest over what cleaners eat. Facultative cleaners would be treated as *Labroides* cleaners and chased in response to jolts even if that chasing might be nonfunctional. If this possibility was confirmed in future studies, the conclusion would be that we must be cautious in using theory to predict the course of interactions in empirical examples (Bshary & Bronstein 2004). Theoretical work on the stability of mutualisms and cooperation has focused on the link between particular game structures and corresponding control mechanisms (reviewed in Bshary & Bronstein 2004; Sachs et al. 2004; Foster & Wenseleers 2006; Bergmüller et al. 2007; Bshary &

Bergmüller 2008) but our study suggests that species may often lack the necessary adaptations to behave as predicted.

On the Origins of Cleaning Mutualisms

Our results are in line with the hypothesis that cleaning mutualism originated as a by-product mutualism (Brown 1983) in which cleaners initially picked small invertebrates from the substrate, which incidentally also made them pick food on other fishes. This could have set the stage for the coevolution of more complex behavioural interactions (Bergstrom et al. 2003) in which the clients were selected to seek cleaners and signal to them their need to be served because of the benefits of parasite removal (Côté 2000). Our results suggest that selection has favoured more sophisticated strategies that involve cheating and manipulation of clients only in cleaner species that increasingly specialized in cleaning as a means to gain food. However, this possibility needs to be treated with caution, as the two strategically behaving wrasse species in our study are also phylogenetically close. This problem cannot be easily resolved by studying more species because tactile stimulation and strategic behaviours seem to be largely absent in other cleaner fishes. For example, even though cleaning gobies of the genus *Elacatinus* are highly dependent on cleaning and evolved similar colour patterns to dependent cleaner wrasses (Cheney et al. 2009), their interactions with clients appear to be rather free of strategic decisions (Soares et al. 2008b). Nevertheless, when phylogenetic constraints are accounted for, three variables show significant effects, suggesting that not all aspects of mutualistic interactions addressed in the present study rely strictly on traits shared by the two closely related, strategically acting species but result from more general ecological rules.

Our results complement well previous studies on the evolution of cleaning mutualism that investigated the role of colour and body size. Apparently, an important feature in the evolution of cleaning behaviour in the labrid family was the presence of a dark lateral stripe (Arnal et al. 2006). While facultative cleaners also often combine a dark stripe with yellow and blue colours, such combinations are particularly frequent in the most dependent cleaner species (Cheney et al. 2009). In contrast to colour, body size measures do not seem to explain the evolution of cleaning behaviour (Arnal et al. 2006), and adaptations in jaw anatomy (Westneat et al. 2005) appear to be derived as a consequence of cleaning. Thus, apparently signals facilitated the evolution of cleaning interactions, which speaks against a parasitic origin. Anatomical and behavioural features of cleaning mutualism appear to be derived: only with increasing dependency have some lineages/species evolved a specialized jaw anatomy for efficient foraging and behavioural strategies to manipulate and exploit their food patches better. In conclusion, the combined evidence favours the hypothesis that by-product benefits were at the origin of the cleaning mutualism. A more broad evaluation of the diversity of multispecies mutualisms should allow us to infer the relative importance of parasitic origins versus by-product benefits in explaining the evolution of mutualism.

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