The skin microbiome

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Abstract | The skin is the human body's largest organ, colonized by a diverse milieu of microorganisms, most of which are harmless or even beneficial to their host. Colonization is driven by the ecology of the skin surface, which is highly variable depending on topographical location, endogenous host factors and exogenous environmental factors. The cutaneous innate and adaptive immune responses can modulate the skin microbiota, but the microbiota also functions in educating the immune system. The development of molecular methods to identify microorganisms has led to an emerging view of the resident skin bacteria as highly diverse and variable. An enhanced understanding of the skin microbiome is necessary to gain insight into microbial involvement in human skin disorders and to enable novel promicrobial and antimicrobial therapeutic approaches for their treatment.

The skin is an ecosystem composed of 1.8 m^2 of diverse habitats with an abundance of folds, invaginations and specialized niches that support a wide range of microorganisms.

The perception of the skin as an ecosystem — composed of living biological and physical components occupying diverse habitats — can advance our understanding of the delicate balance between host and microorganism. Disruptions in the balance on either side of the equation can result in skin disorders or infections.

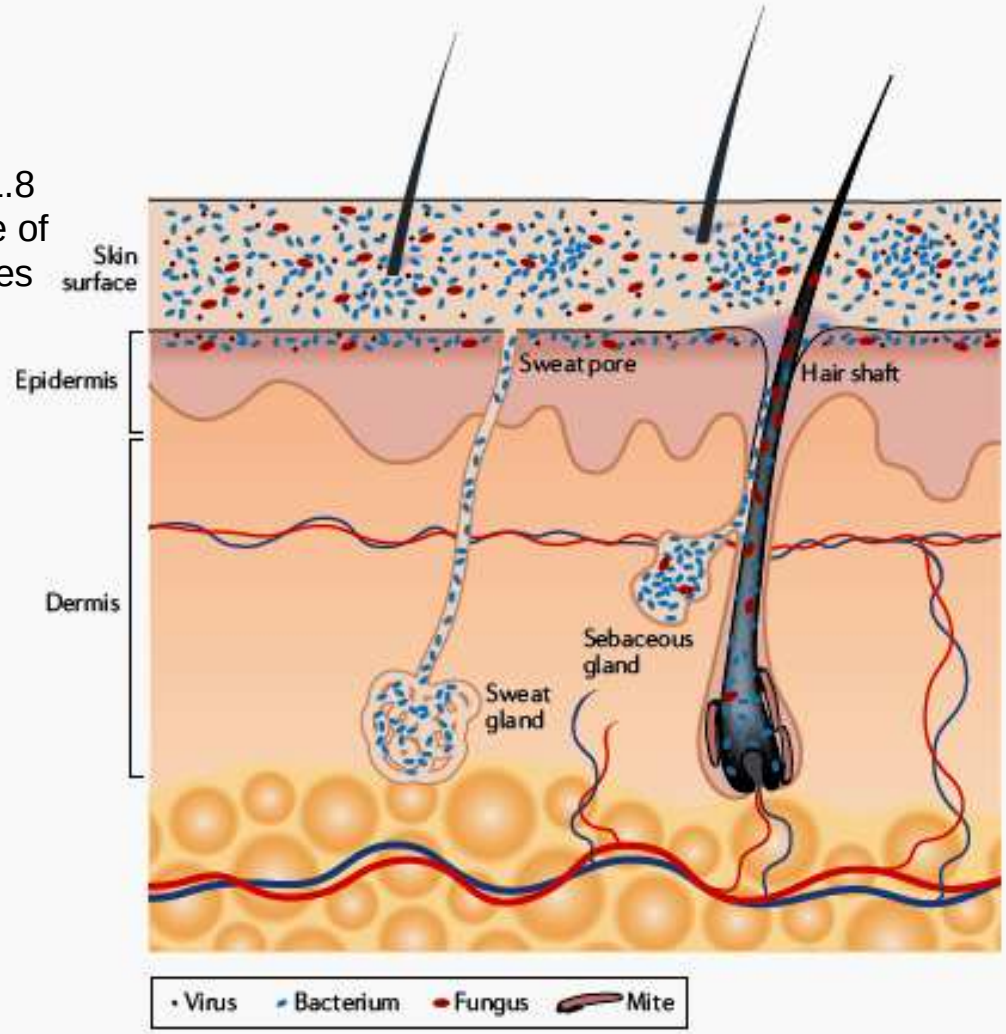


Figure 1 | **Schematic of skin histology viewed in cross-section with microorganisms and skin appendages.** Microorganisms (viruses, bacteria and fungi) and mites cover the surface of the skin and reside deep in the hair and glands. On the skin surface, rod and round bacteria — such as Proteobacteria and *Staphylococcus* spp., respectively — form communities that are deeply intertwined among themselves and other microorganisms. Commensal fungi such as *Malassezia* spp. grow both as branching filamentous hypha and as individual cells. Virus particles live both freely and in bacterial cells. Skin mites, such as *Demodex folliculorum* and *Demodex brevis*, are some of the smallest arthropods and live in or near hair follicles. Skin appendages include hair follicles, sebaceous glands and sweat glands.

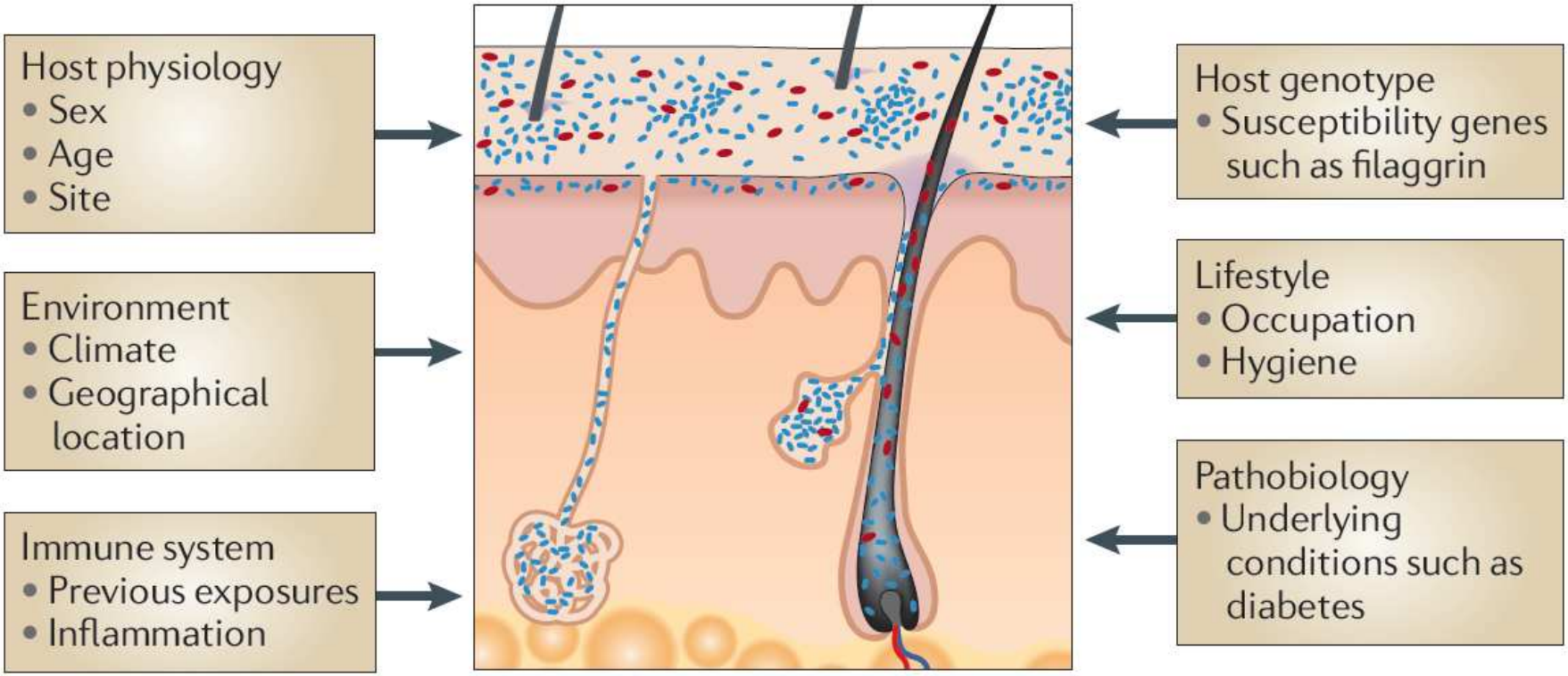
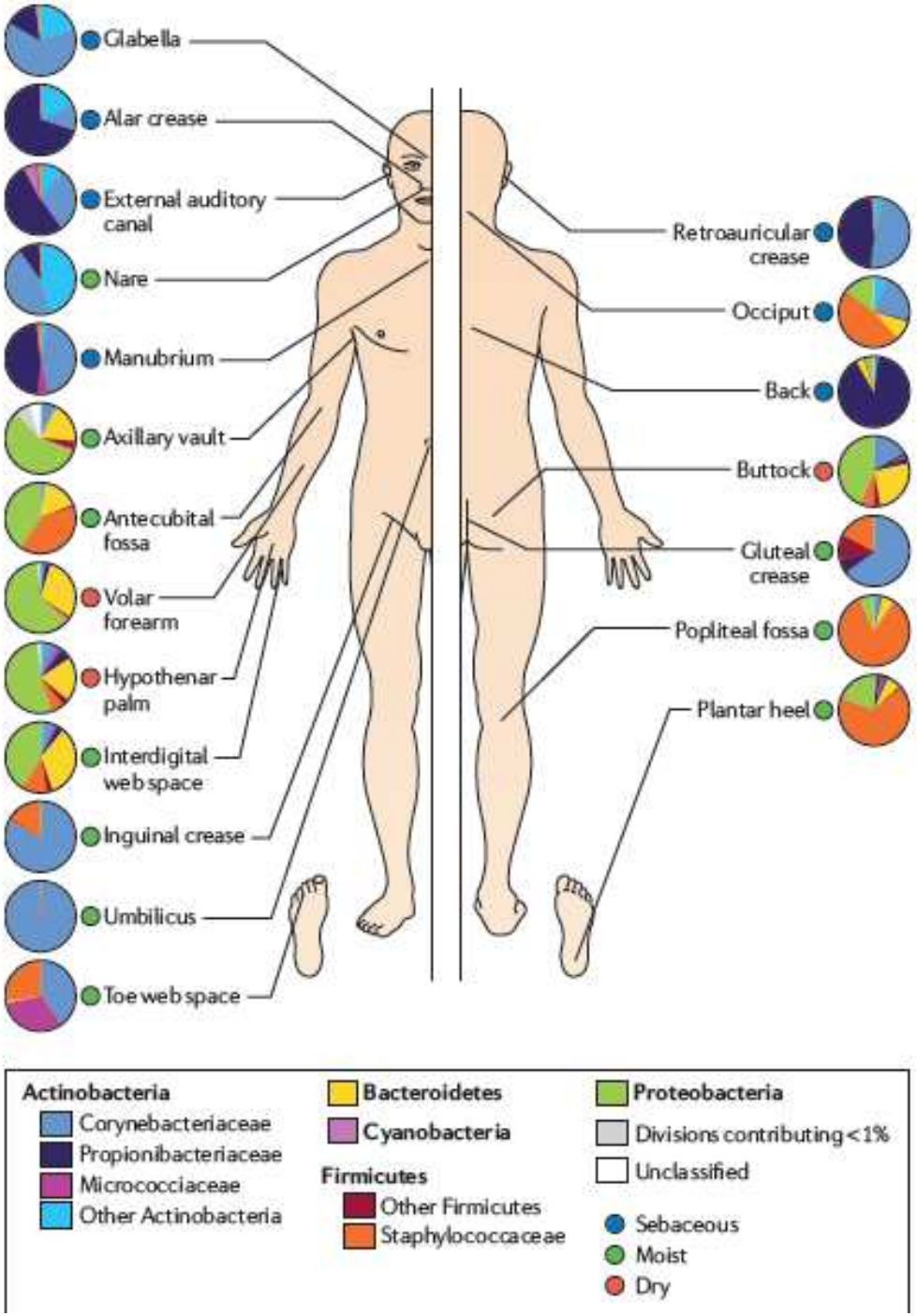


Figure 2 | **Factors contributing to variation in the skin microbiome.** Exogenous and endogenous factors discussed in this Review that contribute to variation between individuals and over the lifetime of an individual.

Sebaceous glands are relatively anoxic and support the growth of facultative anaerobes such as *Propionibacterium acnes*, a common skin commensal bacterium3,13. Full genome sequencing of *P. acnes* has revealed multiple genes encoding lipases that degrade skin lipids of sebum19. *P. acnes* hydrolyses the triglycerides present in sebum, releasing free fatty acids onto the skin20,21. The bacterium can then adhere to these free fatty acids, and this perhaps aids in the colonization of the sebaceous gland22. These free fatty acids also contribute to the acidic pH (~5) of the skin surface4,10. Many common pathogens, such as *Staphylococcus aureus* and *Streptococcus pyogenes,* are inhibited by an acidic pH, thus the growth of coagulase-negative staphylococci and corynebacteria is favoured10,23–25. However, skin occlusion results in an elevated pH, which favours the growth of *S. aureus* and *S. pyogenes*24. Because humans produce much greater quantities of triglyceride-containing sebum than other mammals, *P. acnes* is present in greater abundance on human skin than on the skin of other mammals26

Topography. The skin surface varies topographically owing to regional differences in skin anatomy and, according to culture-based studies, these regions are known to support distinct sets of microorganisms. Some regions of the skin are partially occluded, such as the groin, axillary vault and toe web. These regions are higher in temperature and humidity, which encourages the growth of microorganisms that thrive in moist conditions (for example, Gram-negative bacilli, coryneforms and *S. aureus*). The density of sebaceous glands is another factor that influences the skin microbiota, depending on the region. Areas with a high density of sebaceous glands, such as the face, chest and back, encourage the growth of lipophilic microorganisms (for example, *Propionibacterium* spp. and *Malassezia* spp.). Compared with other skin sites, arm and leg skin is relatively desiccated and experiences large fluctuations in surface temperature. Using culture-based methods, these areas were found to harbour quantitatively fewer organisms than moist areas of the skin surface.



Variation by:
skin site
temporal variations
by individuals

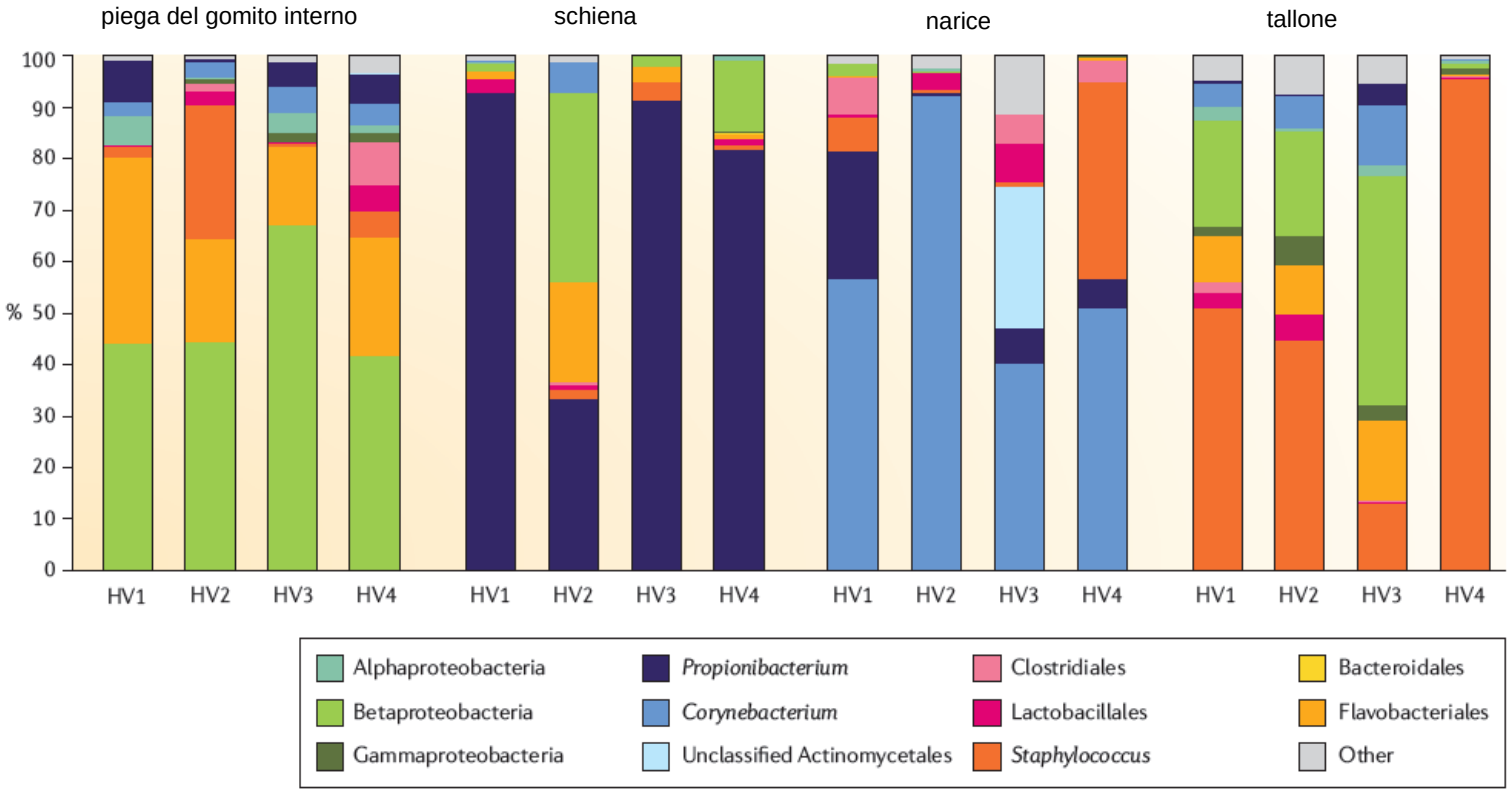


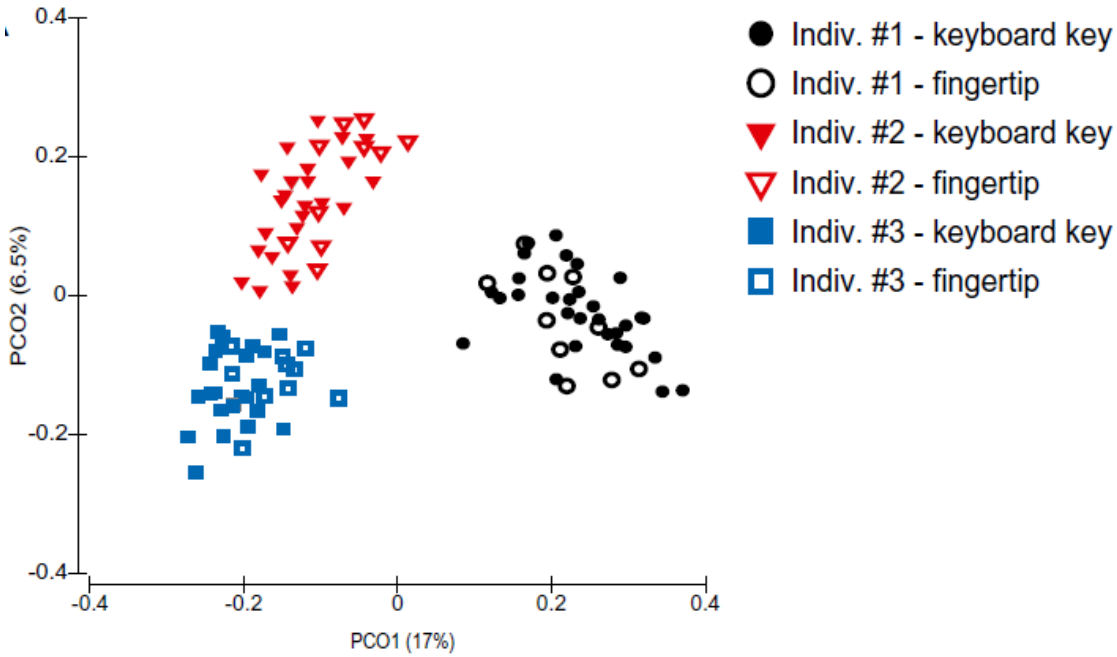
Figure 4 | **Interpersonal variation of the skin microbiome.** The microbial distribution of four sites on four healthy volunteers (HV1, HV2, HV3 and HV4) is depicted at the antecubital fold (inner elbow; part **a**); the back (part **b**); the nare (inside the nostril; part **c**); and the plantar heel (bottom of the heel of the foot; part **d**). Skin microbial variation is more dependent on the site than on the individual. Bars represent the relative abundance of bacterial taxa as determined by 16S ribosomal RNA sequencing. Data from REF. 42.

Whole-genome shotgun
metagenomic sequencing

Forensic identification using skin bacterial communities

We show that skinassociated bacteria can be readily recovered from surfaces (including single computer keys and computer mice) and that the structure of these communities can be used to differentiate objects handled by different individuals, even if those objects have been left untouched for up to 2 weeks at room temperature.

First, we compared bacterial communities on individual keys of three computer keyboards to the communities found on the fingers of the keyboard owners. Second, we examined the similarity between skin-associated bacterial communities on objects stored at −20 ℃ (a standard method for storing samples before DNA extraction) versus those objects stored under typical indoor environmental conditions for up to 14 days. Finally, we linked objects to specific individuals by comparing the bacteria on their computer mice against a database containing bacterial community information for more than 250 hand surfaces, including the hand of the owner.



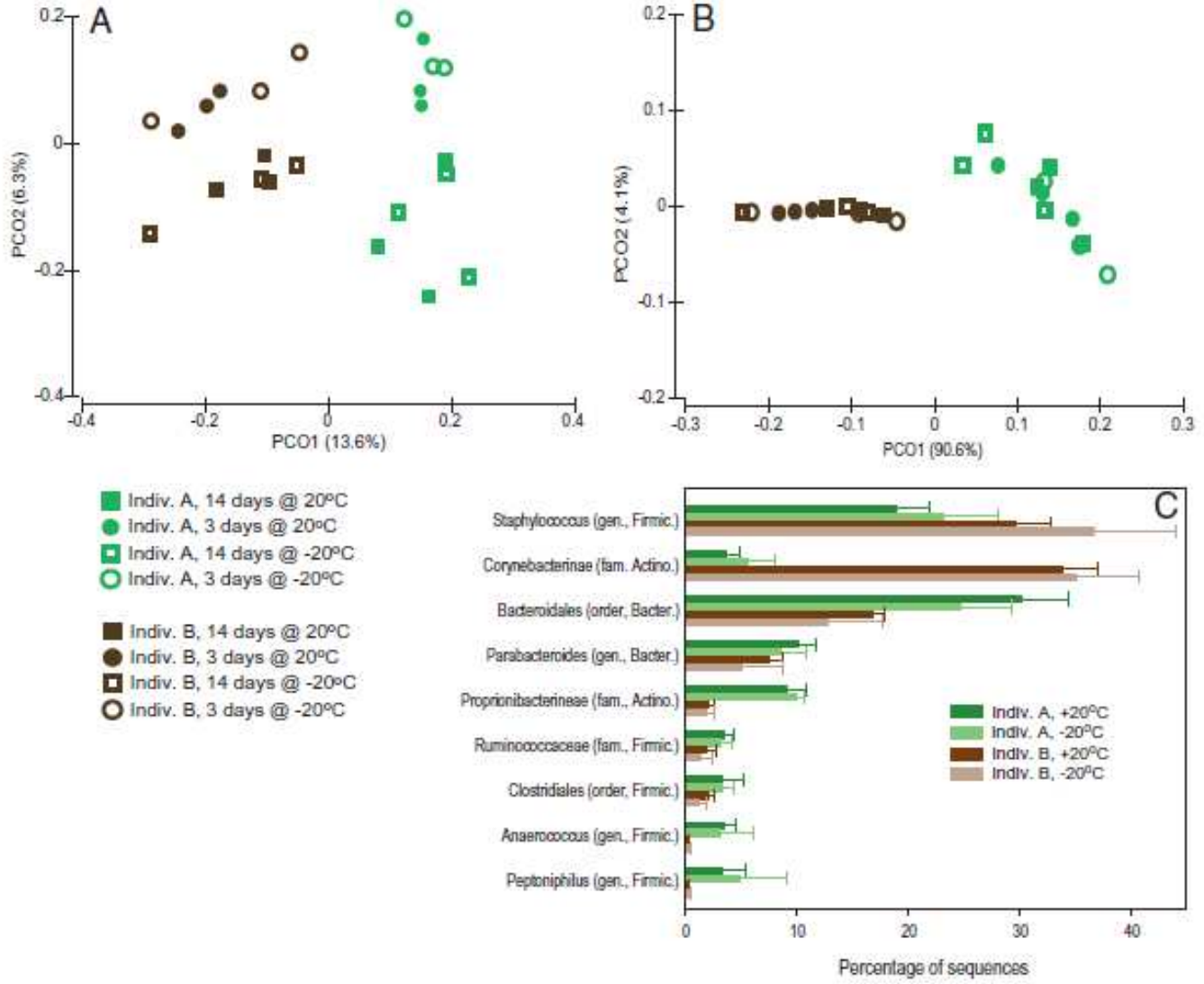


Fig. 3. Effect of storage conditions on skin-associated bacterial communities collected on dry cotton swabs. (*A* and *B*) Principal coordinates plots generated using the unweighted and weighted UniFrac distance matrices, respectively. Samples were stored at either −20 °C or +20 °C with DNA extracted from the swabs after 3 days and 14 days, but storage temperature had minimal effects on bacterial community composition. (*C*) Relative abundances of the most abundant bacterial taxa after 14 days at either −20 °C or at +20 °C. Classifications are to the genus (gen.), family (fam.), or order level. For each taxon, the phylum or subphylum is also indicated: Actino., Actinobacteria; Bacter., Bacteroidetes; Firmic., Firmicutes. Taxa are classified to the highest taxonomic level to which they could be confidently assigned.

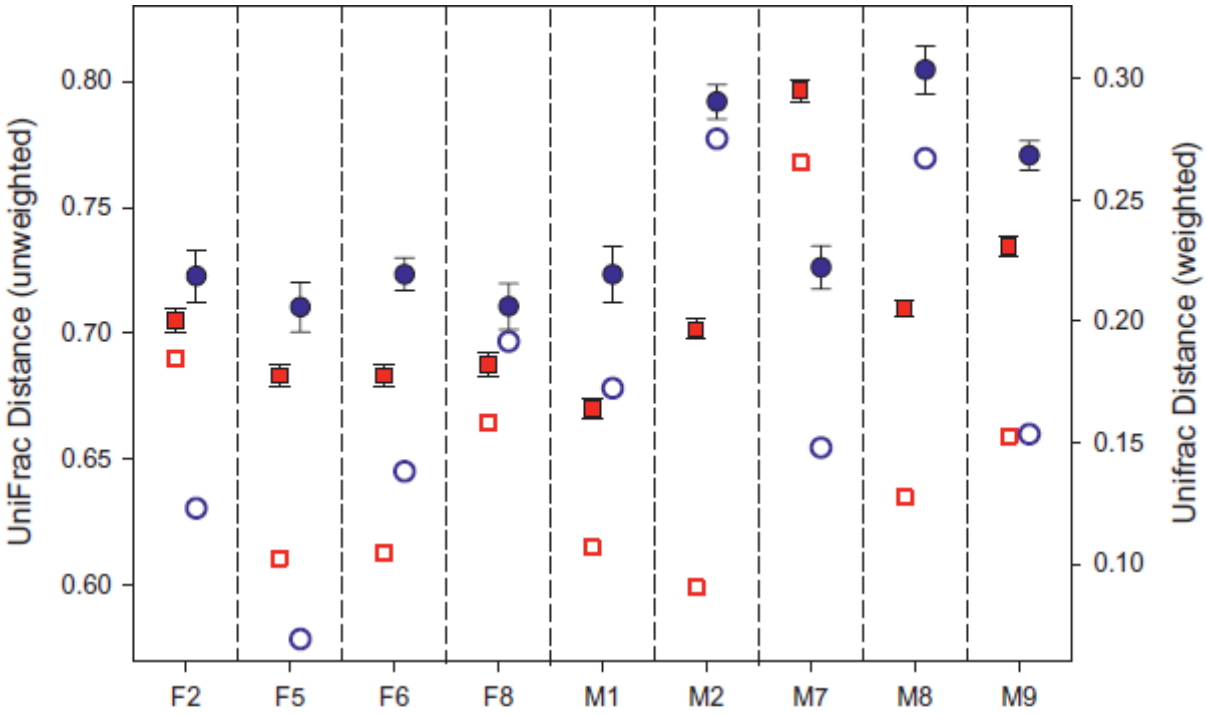


Fig. 4. Accuracy of forensic identification using bacterial communities. Phylogenetic distance between the bacterial communities found on the computer mouse (with the nine mice identified with the *x* axis labels) and the hand swab from the individual that used the mouse (the unfilled symbols) versus the average phylogenetic distance between the bacterial communities on the computer mouse and the 270 other hand swab samples in the database (filled symbols). Error bars represent 95% confidence intervals. Phylogenetic distance measured using either the unweighted or weighted UniFrac algorithm (red squares and blue circles, respectively); the more similar the communities the lower the distance. Note that in nearly all cases the bacterial community on a given mouse is significantly more similar to those on the owner's hand than to the other hands in the database.

Skin MIcrobiome and disease

A causative microbial component that fully satisfies Koch's postulates has rarely been identified in these skin diseases

Skin disorders with correlation to microbiota

- **Seborrhoeic dermatitis (***Malassezia* spp)

As *Malassezia* spp. are present on healthy skin, and by themselves are not sufficient to cause seborrhoeic dermatitis, other factors probably contribute to their pathogenicity and ability to cause disease.

- **Acne**

The commensal skin bacterium *P. acnes* is associated with the very common teenage malady acne, an inflammatory disorder of the pilosebaceous unit.
The onset of puberty matures the pilosebaceous unit, increasing the preponderance of lipophilic microorganisms, especially *P. acnes,* which secretes lipases, proteases and hyaluronidases that injure the tissue lining of the pilosebaceous unit. The *P. acnes* genome encodes various immunogenic factors, including cell surface proteins with adherent properties and porphyrins. Furthermore, the damage caused by *P. acnes* in the pilosebaceous unit activates the classical and alternative complement pathways and induces production of pro-inflammatory cytokines

AD is a chronic relapsing disorder that affects ~15% of US children and ~2% of adults, and is also associated with microbial colonization and infection. The prevalence of AD has doubled or tripled in industrialized countries over the past three decades with no clear cause.
The most common treatments for AD include topical or systemic antibiotics, and steroids**.** (*S. aureus*)

Skin disorders with an unidentified microbial component

Chronic wounds, affecting diabetic, elderly, and immobile individuals, are an example where commensal skin organisms invade and become pathogenic upon breach of the skin barrier. Although bacteria do not cause the initial wounding event.
Burn wounds commonly become infected with *S. pyogenes*, *Enterococcus* spp. or *Pseudomonas aeruginosa*, and can also become infected with fungi and/or viruses

S. epidermidis: ***an invasive skin commensal that causes infection.***
The third category is that of skin microorganisms that are normally commensal but that can sometimes cause infection and disease, especially when they invade other sites. *S. epidermidis* is a very common skin commensal, but it is also the most frequent cause of hospital-acquired infection on in-dwelling medical devices such as catheters or heart valves. After they gain entry, virulent strains of these organisms can form biofilms on catheters or other devices, which protects them from the host immune system and antibiotics. Increasing levels antibiotic resistance, particularly to oxacillin or methicillin, complicates treatment of *S. epidermidis* infections. Furthermore, *S. epidermidis* seems to be a reservoir of antibiotic-resistance genes that it transfers to the closely related but more virulent organism, *S. aureus*

(biocide triclosan resistance *fabI* allele)

Skin MIcrobiome and Allergy

Environmental biodiversity, human microbiota, and allergy are interrelated

According to the "**biodiversity hypothesis**," reduced contact of people with natural environmental features and biodiversity may adversely affect the humancommensal microbiota and its immunomodulatory capacity.

the **"biodiversity hypothesis"** proposes that reduced contact of people with natural environmental features and biodiversity, including environmental microbiota, leads to inadequate stimulation of immunoregulatory circuits

Analyzing atopic sensitization (i.e., allergic disposition) in a random sample of adolescents living in a heterogeneous region of 100 × 150 km, we show that environmental biodiversity in the surroundings of the study subjects' homes influenced the composition of the bacterial classes on their skin. Compared with healthy individuals, atopic individuals had lower environmental biodiversity in the surroundings of their homes and significantly lower generic diversity of gammaproteobacteria on their skin.

The functional role of the Gram-negative gammaproteobacteria is supported by in vitro measurements of expression of IL-10, a key anti-inflammatory cytokine in immunologic tolerance, in peripheral blood mononuclear cells. In healthy, but not in atopic, individuals, IL-10 expression was positively correlated with the abundance of the gammaproteobacterial genus Acinetobacter on the skin.

Composition of Human Skin Microbiota Affects Attractiveness to Malaria Mosquitoes

The African malaria mosquito Anopheles gambiae sensu stricto continues to play an important role in malaria transmission, which is aggravated by its high degree of anthropophily, making it among the foremost vectors of this disease. In the current study we set out to unravel the strong association between this mosquito species and human beings, as it is determined by odorant cues derived from the human skin. **Microbial communities on the skin play key roles in the production of human body odour**. We demonstrate that the **composition of the skin microbiota affects the degree of attractiveness of human beings to this mosquito species**. Bacterial plate counts and 16S rRNA sequencing revealed that individuals that are highly attractive to **An. gambiae s.s. have a significantly higher abundance, but lower diversity of bacteria on their skin than individuals that are poorly attractive**. Bacterial genera that are correlated with the relative degree of attractiveness to mosquitoes were identified. The discovery of the connection between skin microbial populations and attractiveness to mosquitoes may lead to the development of new mosquito attractants and personalized methods for protection against vectors of malaria and other infectious diseases.

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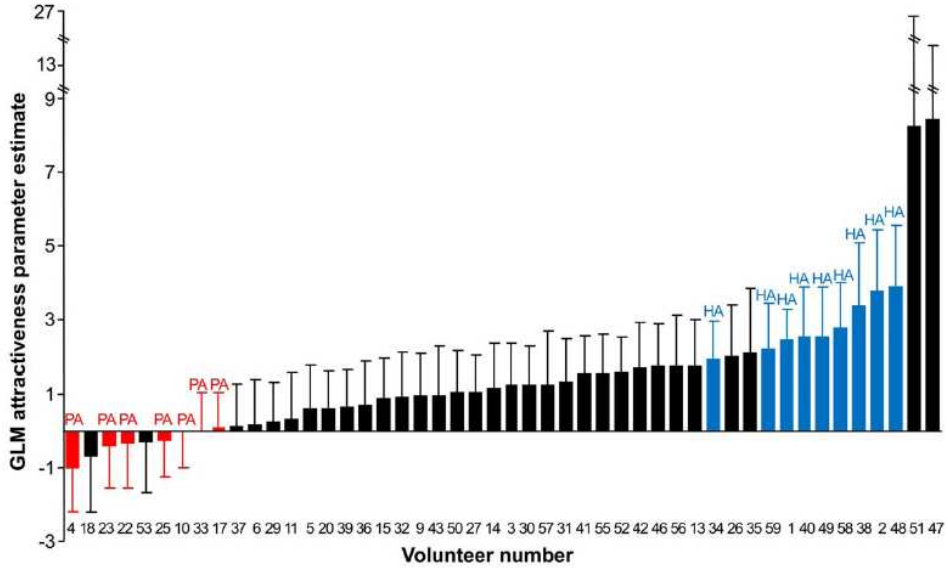

Figure 1. Relative attractiveness to *An. gambiae* of 48 individuals. Bars show the attractiveness parameter estimate results Generalized Linear Model (GLM) used to investigate the relative attractiveness [9] of each individual to *An. gambiae*. Individuals were cl highly attractive (HA, blue bars) when their mean relative attractiveness was significantly higher than the mean relative attractivenes individual in the group classified as poorly attractive (PA, red bars) (GLM, $p<0.05$). Error bars represent the standard error of the mea replications.

doi:10.1371/journal.pone.0028991.g001

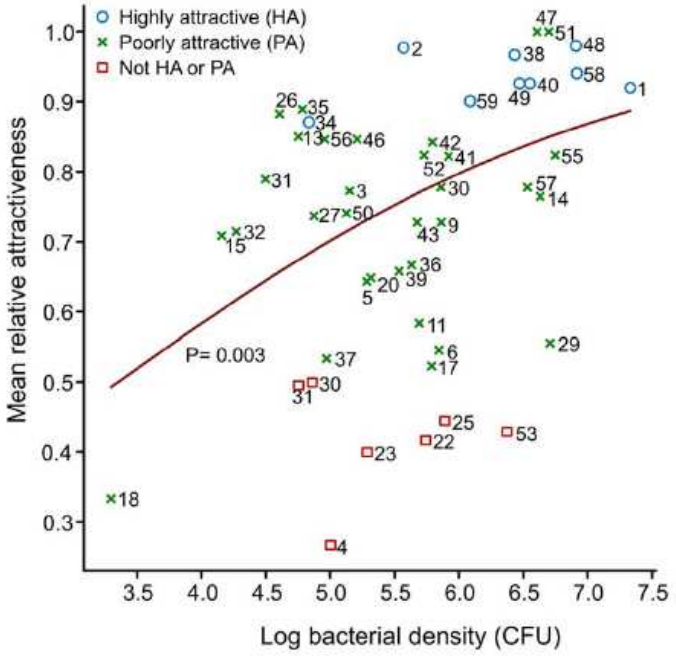

Figure 2. Skin bacterial abundance and relative attractiveness to *An. gambiae*. Correlation between the number of bacteria (log), determined by counts of colony forming units (CFUs) on non-selective plates and the relative attractiveness of the individuals. The relative attractiveness is expressed as the number of mosquitoes caught in the trapping device releasing the odour of the tested individual divided by the total number of mosquitoes trapped in both trapping devices [9]. The red line indicates the fitted relationship according to the Generalized Linear Model (GLM).

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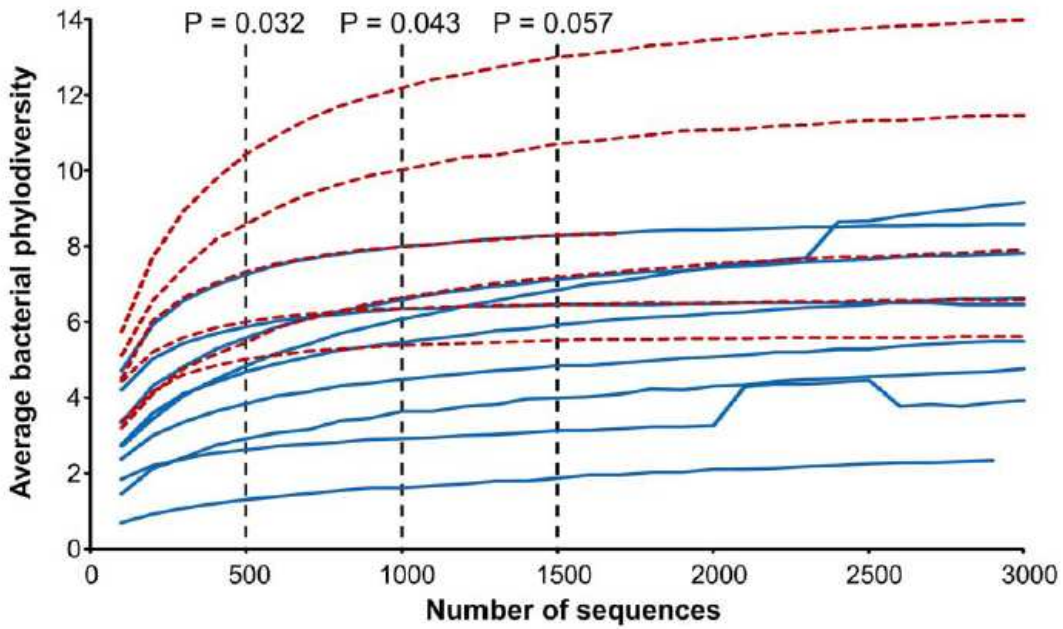
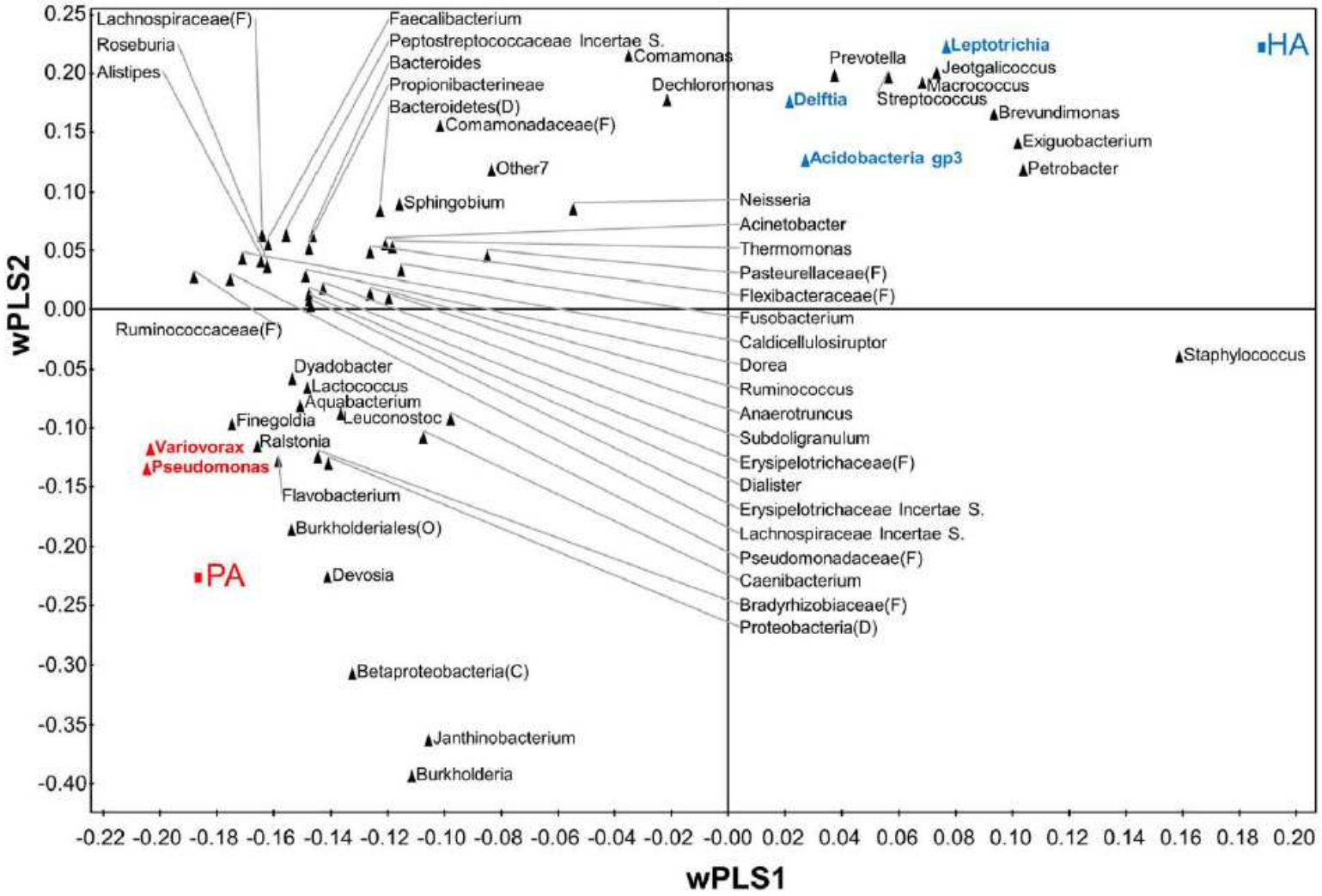


Figure 3. Rarefaction curves showing average bacterial diversity from poorly attractive (PA,) and highly attractive (HA) individuals. *P*-value for the difference in diversity score between PA (dashed red line) and HA (solid blue line) individuals is given at three sampling depths (not calculated for higher numbers of sequences, because the samples from some individuals did not yield more than 1500 sequences).
doi:10.1371/journal.pone.0028991.g003



Only a small part of the bacteria found on the human skin are culturable and therefore it was an important confirmative finding that the results from our in vivo study corroborated previous in vitro studies in which volatiles released by *Staphylococcus epidermidis* were attractive to An. gambiae females and volatiles from *Pseudomonas aeruginosa* unattractive